TEXT IS FLY

UNIVERSAL
LIBRARY

MICROCHEMICAL LABORATORY MANUAL

MICROCHEMICAL LABORATORY MANUAL

BY

FRIEDRICH EMICH

DR. PHIL. H. C., DR. ING. E. H.

Professor at the Technische Hochschule of Graz

W. Mitglied der Akademie der Wissenschaften, Vienna

WITH A SECTION ON

SPOT ANALYSIS

By DR. FRITZ FEIGL

Privatdozent at the University of Vienna

TRANSLATED BY

FRANK SCHNEIDER, SC.M.

NEW YORK

JOHN WILEY & SONS, INC.

LONDON: CHAPMAN & HALL, LIMITED

1932

PRINTED IN U. S. A.

PRESS OF
BRAUNWORTH & CO., INC.
BOOK MANUFACTURERS
BROOKLYN, NEW YORK

TRANSLATOR'S PREFACE

AT THE present time, the term "microchemistry" brings to the minds of many English-speaking chemists quantitative organic microanalysis. The earlier and more comprehensive work of Professor Emich and his coworkers is comparatively unknown. In the opinion of the translator, this fact is partly due to the wide publicity given the organic analysis as a result of the awarding of the Nobel Prize in chemistry to Professor Pregl, and partly, perhaps chiefly, to the lack of an adequate text on the subject in English. It is to supply the latter want that the present volume is published.

Courses of instruction in microchemical methods are now being given by several colleges and universities in this country. This book is designed not only to serve as a text for such courses, but it was also the intention of the author to provide for self-instruction by the student to whom such courses were not available. In view of these facts, the translator has endeavored to attain maximum clarity even at the expense of brevity while retaining as far as possible the original construction.

The translator has been working in this field for the past few years with Dr. A. A. Benedetti-Pichler, former assistant and coworker of Professor Emich. It is his hope that this contact, together with the invaluable assistance given him by Dr. Pichler, has enabled him to translate the "Manual," if at times not literally, still in the sense and spirit of the author.

Our experience gained in teaching the subject has demonstrated that microchemistry is an agreeable change for the student from the usual routine work. The experiments call for skill and ingenuity on his part, and are of great assistance in developing that "chemical asepsis," as it might be termed, which is of such great value in his later work.

With respect to the literature references and sources of supply of apparatus, American sources have been given in addition to the ones cited in the original. Some of the newer papers on the subject, such as the qualitative and quantitative electrolytic methods, which were published after the German edition went to press, have been included. All these additions have been made with the consent of Professor Emich. Numerous suggestions were made by American friends during

the course of the work of translation and were adopted to as great an extent as possible.

It is the earnest hope of the translator that this American edition not only will aid those who wish to make use of these ingenious methods but also will be a tribute to that grand "old master" of microchemistry, Professor Friedrich Emich.

FRANK SCHNEIDER.

New York, November, 1931.

FROM THE FOREWORD TO THE FIRST EDITION

THE publication of the present little book may be justified for several reasons. First is the increasing interest in general that *micromethods*, particularly in chemistry and biology, have aroused, and secondly, the difficulties encountered at present, especially by the chemist, in obtaining material, compelling him to work with small quantities. I believe that the time is not far distant when the majority of our younger colleagues will be required to have some acquaintance with these methods. This, of course, does not mean that micromethods will displace macromethods. The two should be employed in conjunction to a greater degree, as it is to the interest of the utmost economy that each method be employed where it can best be utilized. But it often happens that a problem can be solved by means of micromethods which otherwise would require a disproportionately large expenditure of material, time and energy. The chemist who does not employ these methods, neglects a very valuable tool.

As concerns *instruction* in microanalysis, it appears to me to be immaterial whether it is carried out separately or incorporated in existing courses. W. Böttger has, in his "Qualitative Analysis," [1] followed the second method, but naturally, considering the purpose of the book, only a few of the micromethods could be discussed. Many contemporary analytical books give scarcely any consideration to this field, and since no text which discusses approximately the entire field has been published (since the edition of my "Lehrbuch" [2] has been exhausted) I have gladly given heed to the request of the publisher, that I write a book on microchemical practice. . . .

As in the "Lehrbuch," I have not considered in the "Manual" the related fields for the discussion of which I do not consider myself qualified, particularly the botanical, mineralogical, biological and pharmaceutical phases, as also radiochemistry and metallography.

This little volume endeavors to enable the student to cover the entire program in the daily allotment of work of one semester. It is understood that a training in qualitative and quantitative chemical analysis and in preparative work is an absolute prerequisite. If these prelimi-

[1] Third edition, Leipzig, 1913.
[2] Wiesbaden, 1911.

naries have been complied with and the program carried out with the necessary conscientiousness, I hope to be able to bring the student to the point where he can proceed even when no definite plan is at his disposal.

However, if one is not in a position to devote much time to the subject, an abridged program will suffice. In order to facilitate the selection of the work, two type sizes have been used in the printing.

On glancing through this book, the director of an institute may feel *that he is not in a position to permit microchemical work, as the necessary facilities are lacking. I believe that in general such fears are not justified.* Much can (and should) be made by the student himself, some can be made by the mechanician of the institute, so one can manage, in the beginning at least, with little of the ability of an inventor. When the advantages of the microchemical methods have become apparent, the decision to acquire the necessary equipment will be made more readily, the outlay therefor being offset by the possibility of future savings.

Regarding the sources from which this little volume has been compiled, I can say briefly that all the literature appearing prior to 1911 was given consideration in the "Lehrbuch." Many of the references in question are also included in the "Manual." Among the publications appearing subsequently, I have utilized Pregl's "Microanalysis," and I hereby thank my honored colleague for the permission to make use of his book. To my friend, Professor Dr. N. Schoorl (Utrecht), whose "Contributions" [3] also proved of great assistance, I am under obligation for many suggestions. Finally, my assistant, Dr. Benedetti-Pichler, is to be heartily thanked for his sincere cooperation.

F. EMICH.

Graz, April, 1923.

[3] Z. anal. Chem. **46, 47, 48.**

FOREWORD TO THE SECOND EDITION

THE fact that the first edition of the present little book was sold out in a relatively short time clearly demonstrates that a need for such a compilation exists. To be sure, the situation has changed slightly since the first appearance of the "Manual," in so far as at that time (1924) the "Lehrbuch" was no longer obtainable but has now appeared in a new edition.[1] In spite of this, I believe that simultaneous publication of *both* books is justified. The "Manual" is designed primarily for use at the work bench; the student who in general cannot readily practice microanalysis to any great extent in addition to his other duties should get a *general idea* of the most important microchemical *methods* and by means of simple oft-tested experiments be able to determine what can be accomplished in chemistry by such methods. If the student has advanced so far, finds pleasure and interest in this field and attains the desired skill, he may progress further with the aid of the "Lehrbuch" and the other already very comprehensive literature.

Naturally several abridgments have been made in this connection (particularly as concerns the literature references), but some innovations have been introduced in their stead. Especially has my colleague, Dr. Fritz Feigl, placed, at my request, a contribution on spot analysis at my disposal. Furthermore, schlieren experiments, our new fractionation and crystallization methods and much else are taken up.

I am indebted to many colleagues for kind suggestions, especially to W. Böttger (Leipzig), H. Brintzinger (Jena), E. M. Chamot (Ithaca, N. Y.), A. Friedrich (Vienna), G. Lunde (Stavanger), Ad. Mayrhofer (Vienna), Hans Meyer (Prague), M. Nicloux (Strasbourg), L. Rosenthaler (Berne) and N. Schoorl (Utrecht). I would have gladly acceded to *all* of the wishes expressed, but unfortunately this was impossible in view of the available space. In any case, all the gentlemen are warmly thanked for their friendly interest.

I likewise owe heartiest thanks to my zealous coworkers: Privatdozent Dr. Anton Benedetti-Pichler (at present in New York), Dr. Herbert Alber and Dr. Edgar Schally. The experience acquired by them was gained largely in our guest courses, that is, in association with

[1] Munich, 1926.

chemists some of whom were carrying out microchemical experiments for the first time. Indeed, it is partly due to this fact that the methods in use at our Institute are allotted a larger space.

F. EMICH.

Graz, October, 1930.

CONTENTS

MICROCHEMICAL LABORATORY MANUAL

Introduction

1. The purpose of micromethods is to work with small amounts of substance. What is understood by "small" depends upon the particular case. In botanical microchemistry (which is not included in this presentation) only microscopically small quantities are employed; in qualitative microanalysis "drops" or "droplets" containing, e.g., about 0.001 mg. of active substance are fully adequate; quantitative microanalysis is usually within the range of 2 to 10 mg. of substance, and still higher are the limits of preparative micromethods in which one endeavors as a rule to obtain 1 cg. More details will be given later.

The reasons which compel the chemist to work with small quantities of substance are varied. Some are obvious, such as the purely economical ones. Instead of discussing the matter at greater length, the oft-recurring example may be cited where in an analysis one comes upon traces for the detection of which macromethods fail and micromethods must be used. Moreover, cases also occur where micromethods, because of their simplicity, reliability or speed are superior to the macromethods.[1] Riesenfeld and Schwab have pointed out the value of micromethods in the investigation of *dangerous* substances. One is often able "to control substances . . . whose explosiveness has, up to now, caused all scientists to recoil from their investigation."[2]

2.[3] "The 'sensitivity' of an analytical reaction was for a long time a very distorted conception. The smallest amount of a substance which can be identified, that a substance is still detectable in greatest dilution, that it can be recognized in the presence of large amounts of other substances—any one of these conditions could give a reaction the designation 'most sensitive' without its being characterized any further.

"F. Feigl deserves the credit for his great service in giving every different meaning of sensitivity a definite designation,[4] namely:

Limit of identification for the quantity sensitivity,
Limit of sensitivity for the concentration sensitivity,
Limit proportion[5] for the detectability of x in the presence of A. . . ."

[1] See A. Benedetti-Pichler: Z. angew. Chem. **42**, 954 (1929).
[2] Ber. dtsch. chem. Ges. **55**, 2088 (1922).
[3] Section 2 is taken from the paper of Friedrich L. Hahn, Mikrochem. **8**, 9 (51) **730.**
[4] Mikrochem. **1**, 4 (1923).
[5] This term was originated by Schoorl.

Hahn and Feigl have recently proposed that the term "limit concentration" be used hereafter in place of "limit of sensitivity," with the advantage that the designation "sensitivity" is reserved for application to the whole picture of a reaction. The particular kind of sensitivity to be emphasized depends upon the given case.

The limit of identification is given in γ[6]; the limit proportion is by definition an abstract number. The limit concentration can be given either as the actual concentration (designation $\gamma \times \text{cm.}^{-3} = \gamma$ per cc.) or it can be given as the limit proportion with respect to the solvent (where the number of grams is set equal to the number of cubic centimeters).

According to Feigl,[7] reactions which permit the detection of quantities to $10\,\gamma$ should be considered applicable for microchemical purposes. In this connection the volume of the solution is not considered.

Note: Since the book is primarily intended for practical use, these statements may suffice; in my opinion they express the present state of things. But perhaps I am permitted to draw the attention of the reader to certain points which ought to be considered under the heading "Sensitivity of a chemical reaction."

1. The experimental conditions must be *standardized.* For example, a precipitate may be invisible (one can also say that the reaction was *negative*), but it can be made visible by proper illumination, sedimentation, etc. (and now one can say that the reaction is *positive*).

2. Especially with color reactions a depth of layer must be set. Naturally too great a depth cannot be chosen because of the (eventual) color of the solvent itself and for purely practical reasons. For macro experiments 1 dm. would be suitable, for micro work 1 cm. would be preferable.

3. The suggestion that the limit concentration be introduced as an abstract number has its disadvantages; in practice it is not the same whether I introduce 1γ into 1 cc. or 1 mg. into 1 liter, since in the latter case I can, under certain conditions, make the reaction more easily visible. Therefore the terms γ cm.$^{-3}$ or mg. dm.$^{-3}$ are preferable to the designation 10^{-8}.

4. Finally the question may arise as to whether it is not better to give the sensitivity statements in terms of atomic and molecular or equivalent weights, as has long been done with other numerical terms.[8] In order to avoid excessively large numbers, it would be well to agree upon a system in which exponents are used such as has been introduced in the *p*H values.

3. The book is divided into two parts. In the first (general) part, *apparatus and methods* are described; in the second (specific) part the

[6] $1\,\gamma = 1\ \mu\text{g} = 0.001$ mg. Since the short and convenient term "gamma" is preferred in microchemical publications, we will retain it in the present book. See also Emich, Lehrbuch d. Mikrochemie, Munich, 1926, p. 38, footnote 64.

[7] Cited on p. 1.

[8] See also Böttger, Qualit. Analyse, Leipzig, 1925, p. 622; Emich, Liebigs Ann. **351**, 426 (1907); also Böttger, Mikrochem., Emich-Festschrift 29 (1930).

application to a series of special cases is demonstrated. I assume that the student will read through the general part carefully before he carries out the practice problems. This, of course, requires a few days' time, but gives him a *general view* which provides the foundation one should have before beginning work.

In reference to the individual sections of the specific part the following preliminary remarks may be made.

In the *inorganic qualitative* field "trace detection"[9] especially plays an important rôle; for this one must practice separations as well as individual reactions, the latter particularly as worked out by Behrens.[10] This requires the covering of a wider field from the very beginning. Unfortunately, although trace detection presents great possibilities, it has been so little worked out that we must content ourselves with few references. In most of the examples of separation the methods have been tested for the case *where the ions are present in not too unequal quantities.* An inexperienced analyst will therefore naturally consult in important cases, besides the "Manual," e.g., Böttger's text on qualitative analysis,[11] and *acquaint himself with the behavior of mixtures similar to those in the problem to be solved.*

The modern endeavor to simplify qualitative procedure by the extensive application of *specific* reactions I believe can be given sufficient consideration by applying interspersed examples and by taking up the section on Spot Analysis (F. Feigl). Whether or not the hope of carrying out a qualitative analysis entirely without separations (somewhat similar to spectral analysis) will be realized, remains to be seen. At any rate, the efforts in this direction deserve great attention.[12]

In the *organic qualitative* part, the importance of the individual reactions surpasses that of the separations. In the separations one is of necessity directed to the preparative methods. Therefore these are also considered in this book. Finally the reader's attention is called at this time to Staudinger's "Organic Qualitative Analysis,"[13] in which also the microchemist will find much inspiration.

[9] Emich, Lehrbuch d. Mikrochemie, Munich, 1926, p. V. Gans, Krug and Heuseler, Chem. Centr. 1925 **1**, 829, have noted the inadequacy of the methods used up to now.

[10] Behrens-Kley, Mikrochem. Analyse, Leipzig and Hamburg, 1915 or 1922.

[11] For equivalent English texts see A. A. Noyes, Qualitative Chemical Analysis, 9th ed., Macmillan, New York, 1922; Treadwell-Hall, Analytical Chemistry, 5th ed., John Wiley & Sons, New York, 1919–21; Prescott and Johnson, Qualitative Chemical Analysis, 7th ed., Van Nostrand, New York, 1917.

[12] Kurt Heller, Mikrochemie **7**, 213 (1929); **8**, 33 (1930).

[13] Berlin, 1923. See also N. Schoorl, Org. Analyse (Dutch), Amsterdam, 1920 and 1921. In English: H. T. Clarke, Handbook of Organic Analysis, 4th ed., Longmans Green, New York, 1926; O. Kamm, Qualitative Organic Analysis, John Wiley & Sons, New York, 1923; S. P. Mulliken, A Method for the Identification of Pure Organic Compounds, John Wiley & Sons, New York, 1916.

In the *quantitative* field, I believed several practice examples, which included the most important gravimetric, volumetric and electrometric methods, would be sufficient. The perhaps somewhat stepmotherly treatment of volumetric analysis may be justified by the fact that the most important details of the methods can be learned by acidimetry and the numerous other determinations serve really only very special purposes.[14] Organic quantitative analysis has not been considered.

Likewise, the methods, for example, for the determination of ammonia, acetone, sugar, etc., important in medicine and biology, are not taken up, as I believe that these should be reserved for the special literature. Essentially the same holds for flame tests, spectral reactions, etc.

4. In the exercises, emphasis must be placed upon the fact *that each experiment should be performed flawlessly.* It is better to practice *one* reaction thoroughly so as to master it completely than to carry out a large number superficially. The worker *must* be convinced that the methods can be used, that is, with proper knowledge and ability they will infallibly accomplish their purpose. That a course such as this includes practice with known materials need not be said, nor that practice analyses of materials of unknown composition are to be introduced at the proper places.

5. The lack of certain pieces of apparatus limits the program, to be sure, but does not render micro work impossible. *Every chemist who possesses a microscope can carry out qualitative microanalyses, and where a microbalance is available, quantitative (inorganic) determinations are possible.* Practically all the rest can be improvised more or less easily. In order to facilitate such improvisations we have included several suggestions in Appendix II.

Between this limiting case and an ideally complete outfit all intermediate steps are naturally possible. In order to bring them into a scheme we can distinguish three cases. First, an equipment which is necessary if the course based on the "Manual" is to be carried out more or less completely; second, an equipment which meets somewhat stricter requirements which appear to me to be desirable for e.g., a medium-sized university or college, and third, an equipment which conforms with extensive requirements. These three divisions are indicated in the text as "absolutely necessary," "very desirable" and "desirable."[15]

[14] It would be a praiseworthy task to make a collection of the methods of micro volumetric analysis.

[15] It is said of Faraday that he never regarded an investigation complete until he had applied all the means at his disposal. This principle is certainly excellent for the scientist; in ordinary laboratory work he does not often apply it for external reasons and for reasons of economy, that is, because the increased expenditure of energy and time is not commensurate with the value of the results obtained.—Silvanus P. Thompson, "Michael Faraday's Life and Works."

I. APPARATUS AND METHODS

A. Qualitative Section

I. Microscope and Accessories

(a) General

A *good microscope* is absolutely necessary for qualitative microanalysis; it is also very desirable to have a *simple magnifier* and a *binocular instrument.*

1. Even though it is assumed that the arrangement and use of the compound microscope is, in general, familiar,[1] the following suggestions may be added.

The microanalyst needs primarily a low and a medium magnification; with the former it is desirable to have the greatest possible working distance. One can manage therefore, e.g., with objectives A and D of Zeiss (Jena and New York) or 3 and 6 of Reichert (Vienna),[2] in conjunction with the Huygens eyepieces 2 and 4 of Zeiss and II and IV of Reichert.[3] Thereby, with a tube length of 160 mm., magnifications of (about) 50, 100, 200 and 400 are available.[4] One eyepiece should be equipped with a (removable) micrometer plate; it is desirable to have the second equipped with crosshairs. The microscope should be fitted with a polarization equipment. A high-power eyepiece, a very low-power and a high-power objective and a *rotating* stage are also very desirable.

[1] Recommended and for our purposes generally sufficient are: F. Rinne, Krystallogr. Formenlehre, Leipzig, 1922; A. Kohler, Das Mikroskop und seine Anwendung, Vienna and Berlin, 1923 (Abderhalden's Handbuch der biolog. Arbeitsmethoden, Abt. II, T. I., p. 171); Groth-Jackson, Optical Properties of Crystals, John Wiley & Sons, New York; Chamot and Mason, Handbook of Chemical Microscopy, John Wiley & Sons, New York. The last-mentioned book contains a bibliography of works on this subject.

[2] Reichert objectives are equipped with a cover glass which is mounted directly before the front lens. This protects the lenses of the objective at all times even when no cover glass is used on the preparation itself.

[3] These objectives and eyepieces correspond to objectives 16 mm. and 4 mm. and eyepieces 5× and 10× of Bausch and Lomb (Rochester) and objectives 16 mm. and 4 mm. and eyepieces 5× and 10× of Spencer (Buffalo).

[4] Schoorl (private communication) prefers the 2 and 4c (Reichert) with eyepieces 4 (or 5). The objectives of Zeiss A and D mentioned above carry the new notations 8 and 40, the eyepieces 2 and 4, the notations 5× and 10×.

A displaceable condenser and an iris diaphragm complete the necessary equipment.

The above requirements are fairly well fulfilled in the "small petrographic" stands.[5] If it is at all possible, the acquisition of a larger instrument is very desirable, especially as all later additions can be made directly without further equipment. Such a stand has, of course, a fine and a coarse adjustment and, above all, a complete Abbé illumination apparatus, thereby permitting the use of high magnifications which are necessary, for example, in ultramicroscopic investigations. Furthermore, the larger stands are adaptable to microprojection and photography. The addition of a *revolving objective nosepiece* is very desirable for the sake of saving time. *Objective clamps* are also very convenient. It is inadvisable to permit economy to be the governing factor in the purchase of a microscope.

That the microscope must be protected from acid fumes of the laboratory and above all handled with the greatest care is self-evident. The most dangerous fumes are, of course, hydrogen fluoride vapors which render the lenses worthless. It is recommended that with preparations which evolve such fumes, the "front lens" (i.e., the lens facing the preparation) be protected by means of a drop of water or glycerine laid upon it. In such investigations the high-power lenses are best removed entirely and placed in safety. Objectives with so-called "telescopic" mounting must not be taken apart. For further details see Emich: "Lehrbuch d. Mikrochemie," Munich, 1926; or Chamot and Mason; "Handbook of Chemical Microscopy," Vol. I, New York, 1930.

2. As *simple magnifier* an ordinary pocket lens will serve, or better a Brücke lens with a magnification of 5–10 times. Pregl[6] recommends a watchmaker's lens which can be held in the eye like a monocle and which will be particularly useful to the farsighted. If it happens that such a lens is not available, the eye lens of the low-power eyepiece can serve as a substitute. For the telescopic lens (Zeiss) see page 58.

3. As already stated, it is *very* desirable to acquire a *binocular microscope* constructed according to the Greenough principle, i.e., consisting of two tubes inclined toward each other at a sharp angle. The *erect, relief image makes many tasks extraordinarily easy.* One pair of objectives and perhaps two pair of eyepieces will be sufficient and a magnification of 20 to 50 times will suffice. Recently, "*Stereo*" attachments have been made for the ordinary microscope which also give a relief image.

4. Of primary importance in every magnifying instrument is the proper choice of *illumination,* which must fit the nature of the object.

[5] As a rule, these stands have no fine adjustment, which is inconvenient when working with high magnifications, and the use of very high magnifications is, of course, out of the question.

[6] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930.

During the day a light window space is used; a desk lamp or arc light will serve as a source of artificial light.

Observation under the microscope is usually carried out with " transmitted" light; for this the plane mirror is set so that the light of a white cloud (wall) or light from the sky falls fully on the condenser. (With artificial light [in some cases a candle flame suffices!] a round 2-liter flask filled with water is placed between the light source and the microscope mirror.) The object is examined on a slide either free or imbedded in a suitable liquid, depending upon the nature of the object. With a high magnification a cover glass is always used to prevent the objective from touching the object and to obtain the clearest possible image. With low magnifications the cover glass may be dispensed with in most instances. When focusing with the high powers it is best, for safety sake, to lower the tube with the coarse adjustment until it *almost* touches the cover glass and then raise by means of the adjustment screw until the image is as clear as possible.

If contours, fine lines or drawings are to be observed, the greatest clarity is obtained by varying the opening of the diaphragm. Colored objects are usually observed with a *wide* diaphragm opening and always with inserted condenser.

In the examination of powders which are sorted through in order to pick out a single particle (see Exercise 1), reflected light is preferred as a rule. Since only a low magnification is used, bright daylight or light from a table lamp is sufficient. In special cases a micro arc lamp fitted with a condensing lens is recommended. The change between transmitted light and reflected light is accomplished most simply by holding the hand first in front of the mirror and then between the object and the light source. One should accustom oneself *to test in this manner all objects which are observed with low magnifications.*

For our purposes, reflected light cannot be used with high magnifications. Special devices (vertical illuminators) are necessary in such cases. Often a suitable background of black or white paper laid *under* the slide, will facilitate the observation. The white plate of the (e.g., binocular) preparation microscope does not serve the purpose as well as the above-mentioned paper, for because of its low position, it is usually not illuminated sufficiently.

5. It is best to secure the advice of an experienced microscopist when *testing* the optical and mechanical systems of the microscope.

(b) Measurement of Length under the Microscope

If the true size of a microscopic object is to be determined one makes use of a "micrometer" of which there are different types. The one most frequently used

and which is sufficient for our purposes is the "eyepiece micrometer" in conjunction with the "stage micrometer."

The first is, as known, a round glass plate which has a scale engraved in its center, usually 5 mm. in 0.1-mm. divisions. It can be placed between the eye lens and the field lens (the two lenses of the eyepiece). As a rule the first is adjustable in micrometer eyepieces so that its position can be accommodated to the eye distance of the observer. The micrometer is inserted with the scale downwards. The value of one division is generally given by the manufacturer, but such statements have only an approximate value; one should determine it oneself. To do this, a stage micrometer (slide with engraved scale) is laid on the stage and the eyepiece micrometer calibrated against it. If the stage micrometer is divided into divisions of known value, e.g., a millimeter in 10 or 20 divisions, then direct inspection gives the value of one division of the eyepiece micrometer. A little table should be prepared for the various objectives (and perhaps tube lengths). As unit of length, 0.001 mm. is in general use (1 "micron" = 1μ).

For determination of weight by micrometric measurement, the reader is referred to other works: Emich, "Lehrbuch d. Mikrochemie," p. 20; Chamot and Mason, "Handbook of Chemical Microscopy," Vol. I, p. 422.

(c) The Behavior of Objects in Polarized Light

1. *Double Refraction.* For the study of the phenomena[7] which are considered under this heading, the microscope is equipped with two nicol prisms and a gypsum (selenite) plate. The first nicol, "polarizer," is located below the stage, the other "analyzer" is either inserted between the objective and eyepiece or (rotating) set upon the latter. The gypsum plate can be inserted in different places in the same way anywhere between the polarizer and analyzer.

In the experiments in sections (c) to (e), it is first assumed that the illumination lenses (the condenser) are removed, that is, one works with so-called parallel light.

If we first insert the two nicol prisms and rotate one of them on the axis of the instrument, the field changes in brightness; when it is brightest we call the position of the nicols "parallel," when it is darkest, "crossed." We thereby assume that with crossed nicols the plane of polarization of one nicol passes from back to front and consequently that of the other from left to right [8] (which in most instruments is actually the case or can readily be realized). If a number

[7] It is expressly emphasized that, for the understanding of polarization phenomena, it is necessary to study specialized works of which we may mention, for example, those cited on p. 5, footnote 1. The reader can also find other literature. We must limit ourselves to a short introduction to the use of polarization equipment for the purposes of microchemistry. For simple apparatus see Appendix II.

[8] In order to determine the orientation of the nicols quickly, one can carry out the following experiment. Several needles of anthraquinone are placed in a droplet of nitrobenzene, a cover glass is placed over it and it is observed under the microscope with the nicol in question (polarizer or analyzer) inserted. When the object (or the nicol) is rotated, the needles become pale if the lengthwise direction coincides with the plane of vibration of the nicol (or to whose plane of polarization it is perpendicular). In the position perpendicular to the first, the needles become strongly evident.

of crystals "oriented at random" (so that the crystals are in all possible positions) are brought between crossed nicols, one of two things may occur:

(*a*) The field remains *dark*: crystals which possess this property *in all positions* are called *singly refracting*; they belong to the *cubic* system (sodium chloride). The same behavior is shown by amorphous substances.[9]

(*b*) Some of the crystals will become bright: the crystals are "doubly refracting," they cannot belong to the cubic system but to any other crystal system. Now two cases are possible in which crystals of this type in *definite* positions (in the direction of the so-called "optic axes") behave under these conditions like singly refracting bodies, i.e., do *not* brighten the field.

(α) There is only *one* direction in which a crystal does not brighten when rotated between crossed nicols: the crystal is "optically uniaxial," it belongs to either the *hexagonal* or *tetragonal* systems. The direction of the optic axis coincides in this case with that of the crystallographic principal axis.

(β) There are *two* directions in the crystal which possess the property mentioned above: the crystal is "optically biaxial," it belongs to the *rhombic, monoclinic* or *triclinic* systems.

Under certain conditions it is possible to differentiate between an optically uniaxial and an optically biaxial crystal by means of "converging polarized light." In such cases the observation of so-called axial pictures may supply the information. For details, see, e.g., Emich, "Lehrbuch d. Mikrochemie," p. 23, also section (*e*), p. 10, or Chamot and Mason, "Handbook of Chemical Microscopy," Vol. I, p. 287.

2. Several other properties involving on one hand the "extinction positions" and on the other the "character" of the double refraction are used as marks of identification.

If a doubly refracting crystal is observed between crossed nicols while the stage is slowly rotated through one complete revolution, the crystal will generally appear dark in four positions, which are 90° apart. These positions, in which the crystal behaves like a singly refracting one, are called "extinction positions." In defining extinction positions, the principal edges are usually considered, that is, those by which the crystal is chiefly defined (e.g., in a needle-shaped crystal, the longitudinal edges). These positions can be marked by turning the eyepiece so that they will coincide with the crosshairs. Again, two cases are possible:

(*a*) *Parallel Extinction.* If in the extinction position, the principal edges are parallel, or perpendicular, to the planes of vibration of the nicols (which are at the same time the directions of vibration of the two components of light in the specimen), the extinction is said to be "parallel." This occurs quite often with needle-shaped crystals. Practice preparation: some manganese oxalate (obtained by placing a kernel of oxalic acid in a drop of manganese chloride solution, Fig. 1).

[9] It is assumed here that the material is not influenced in its ability to refract light by pressure through which a sort of artificial double refraction may occur. One also finds frequently with very fine threads and other objects, e.g., starch kernels, phenomena which are similar to double refraction of crystals.

(*b*) *Oblique Extinction.* If the extinction position forms an angle with the planes of vibration of the nicols varying from 0° to 90°, the extinction is said to be "oblique." One can say also briefly "the crystal" has parallel or oblique extinction. By "extinction inclination" is meant the angle which the extinction position forms with the planes of vibration of the nicols. Practice preparation for oblique extinction: sodium chloroplatinate, Fig. 2. The arrows indicate the planes of vibration of the nicols.

(*c*) Occasionally one speaks about "symmetrical extinction," that is, when the crystal is in extinction position, the planes of vibration of the nicols bisect the angle between two adjacent crystal edges.[10]

3. For observation of other characteristics of the doubly refracting crystals, the *gypsum* or *selenite plate* is used. This is of such a thickness and orientated in the microscope so that it shows between crossed nicols a very distinct interference color, "the first order red," which has the property of changing color very easily even if only very weakly doubly refracting crystals are observed together with the selenite plate. The resulting colors are either "addition colors" (addition of retardation) or "subtraction colors" (subtraction of retardation),[11] that is, the object affects polarized light either in the same direction as the selenite plate (addition) or in the opposite (subtraction).

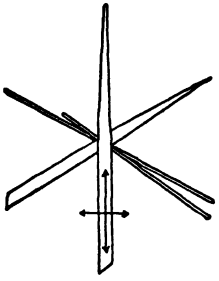


FIG. 1.—Manganese oxalate.

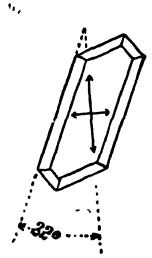


FIG. 2.—Sodium chloroplatinate.

In order to obtain the maximum effect the crystals are tested in a position 45° from that in which extinction takes place; one therefore first turns the stage until the crystal becomes dark and then 45° further. Further details are given in Emich's "Lehrbuch d. Mikrochemie," p. 25, and Mason and Chamot's "Handbook of Chemical Microscopy," Vol. I, p. 280.

4. The polarization equipment of a microscope also serves for the recognition of *pleochroism* (also called "di- or trichroism"). In brief, one understands thereby the difference in color in the different directions of a crystal. This difference can be seen if the crystal is rotated by means of the stage and observed in polarized light (only one nicol used). Of course the crystal may also remain fixed and the nicol turned. With very small crystals this is more advantageous since the eye can detect changes more easily in a stationary object. In this way the two colors which correspond to the directions under consideration are observed *following one another*. The light falling on the object from above or from the side must be cut off during the experiment. Pleochroism is an oft-occurring phenomenon, but is interesting for our purposes only if it is evident to

[10] Chamot-Mason, Handbook of Chemical Microscopy, Vol. I, p. 300.

[11] In the meaning of Schröder v.d. Kolk, Mikr. Krystallbestimmung, Wiesbaden, 1898, p. 26.

a marked degree. Striking demonstration preparations: yttrium platinous cyanide, various quinhydrones, also threads dyed with colloidal metals, etc.

(d) Determination of Refractive Index

1. Optically Isotropic Substances

Any (colorless) object can be seen better, other conditions being the same, the greater the difference between its refractive index and that of its immediate (colorless) surroundings. Therefore one can see a kernel of sodium chloride clearly in air, not so clearly in alcohol and almost not at all in ethylene bromide. We deduce from this that ethylene bromide and sodium chloride have approximately the same refractive index. If in such a case the index of refraction of one substance is known, one can determine that of the other. On this is based the "immersion method," in which the problem is to find a liquid in which the edges of the given object become invisible. For further details and a list of liquids of varying refractive index see Emich's "Lehrbuch d. Mikrochemie," p. 26, and Chamot and Mason's "Handbook of Chemical Microscopy," Vol. I, p. 366. (See also Exercise 3, p. 80.)

2. Optically Anisotropic Substances

If a ray of light enters an optically anisotropic, that is, non-cubic crystal, it is generally broken up into two rays, so it is impossible to speak merely of the index of refraction of an anisotropic crystal. The immersion methods will give results only conditionally free from objection. To be sure, crystal optics teaches that this is possible if *polarized light* is used and the index of refraction obtained in *definite directions* of the crystal. Two values are then obtained which are often characteristic of the substance in question.

The procedure for such cases is given in Exercise 3 on p. 80.

As already remarked, the condenser is removed and the plane mirror used in these experiments.

(e) Characteristic Features for the Identification of the Crystal Systems

Taken from the "Guide to the Determination of Minerals" by Fuchs-Brauns:

"1. All crystals remain dark in every position between crossed nicols; they are singly refracting, *regular* (caesium alum).

"2. Most of the crystals become light and colored between crossed nicols (often only gray) and possess parallel extinction; some remain dark in all positions: they are doubly refracting and optically uniaxial. One must further note the outline of the crystals which remain dark:

"(*a*) The outline of the crystals which remain dark is four-sided (or eight-sided), *square*, the crystals are tetragonal (calcium oxalate).

"(*b*) The outline is six-sided, the crystals are *hexagonal* (sodium fluosilicate).

"(*c*) The outline of the crystals remaining dark is three-sided, the crystals are *rhombohedral* (sodium nitrate).

"3. All crystals become bright (often only gray) and colored between crossed nicols, they are optically biaxial.

"(*a*) All possess parallel extinction, they are *rhombic* (lead chloride).

"(*b*) Most possess oblique, some parallel, extinction, they are *monoclinic* (gypsum).

"(*c*) All crystals show oblique extinction, they are *triclinic* (copper sulfate)."

II. The Receptacles

1. The usual *test tubes* are too large for microanalytical purposes; smaller ones are therefore used. As a rule, however, it is not necessary to go below 1-cc. capacity, that is, 6-mm. width and 30-mm. length. For ordinary work one can make the test tube of soft glass; for exact work, similar tubes of Pyrex or Jena Geräte glass and of quartz are very desirable.[1] They should not have too small a rim.

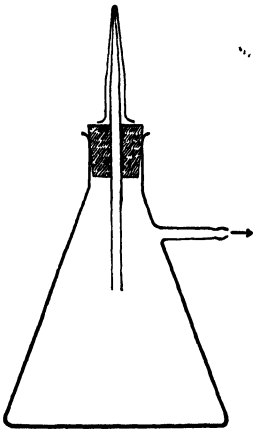


FIG. 3.—Set-up for cleaning centrifuge cones.

2. If the lower half of such a test tube is tapered as in Fig. 3, a *centrifuge cone* will result. In making it, care must be taken not to have the conical part too narrow, as this will render *cleaning* difficult. The cleaning is carried out by the use of the suction apparatus (Fig. 3) connected to a pump; the cone is cleaned by repeated rinsing and inverting over the pointed tube of the suction apparatus. It is dried by waving briefly over a Bunsen flame.

For holding the cones, a wood block (Fig. 4) about 15 by 4 by 3 cm., in which

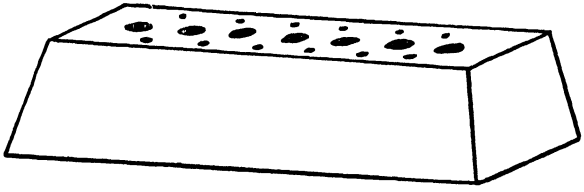


FIG. 4.—Block for holding centrifuge cones.

a number of holes of different diameters are bored, is used. The larger holes hold the test tubes and the centrifuge cones, and the "capillaries" in which a pre-

[1] Quartz tubes may be obtained from the Thermal Syndicate, Ltd., 62 Schenectady Ave., Brooklyn, New York.

cipitate is to settle out, etc., described later, are placed in the smaller ones. Similar blocks are used for microbeakers (Fig. 54).

The centrifuge cone has the advantage over the test tube in that the precipitate is forced together into a small volume and can thereby be observed more easily; also, one can separate it more completely from the solution after sedimentation.[2]

The test drop is not introduced directly into the centrifuge cone from the stock bottle but as a rule by means of a glass (quartz) capillary tube, a platinum loop or stirring hook (p. 23), as only by such means can sufficiently small drops be added.

In the case of colors, the reaction is observed directly with the naked eye against a white background, or possibly as described on p. 92. For the observation of precipitates see below.

3. *Heating* of the test tubes and centrifuge cones is always carried out in a suitable bath, since by open heating, as over a microburner, the liquid is easily thrown out. A device (Figs. 5 and 6) which is laid on the water bath and which can easily be made by tapering a glass tube is used. If the sample is to be evaporated, a stream of filtered air is led into the centrifuge cone by means of the tube shown in Fig. 5. It is often advisable to slip a short piece of rubber tubing over the cone so that only the lower part is heated. For larger vessels, such as microbeakers, the device in Fig. 6 is employed.

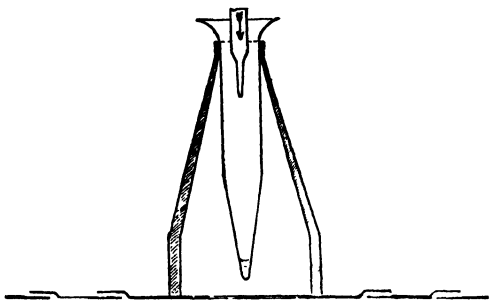


FIG. 5.—Heating centrifuge cones on water bath.

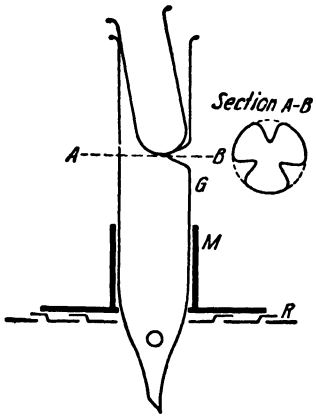


FIG. 6.—Heating microbeakers.
M, Metal plate.
R, Water bath rings.
G, Steam jacket for microbeaker.

To heat *sealed* tubes, either: (*a*) a small *block* of copper or aluminum (see also Exercise 55); or (*b*) a *micro steam bath*, which may be impro-

[2] Essentially the same can be said of the Mayrhofer "capillary test tubes," Mayrhofer, Mikrochemie d. Arzneimittel u. Gifte, Vienna and Berlin, 1923; **1**, p. 6.

vised from a metal test tube, will serve. The author uses iron gas pipes of 13- to 17-mm. diameter and about 18-cm. length which are soldered hard at the lower end. The upper end is slightly widened and filed smooth inside so that the tubes can be tightly stoppered when not in use. It is convenient to have at hand a large number of such tubes with bath liquids of, e.g., toluol (110°), amyl alcohol (130°), anisol (154°), aniline (180°), ethyl benzoate (210°) and quinoline (240°).

(*c*) Ordinary test tubes made of temperature-resistant glass and provided with reflux air condensers are also useful.

(*d*) At this point the reader should be reminded of the enormous pressure which *small* sealed tubes can withstand. They are therefore recommended, e.g., for the demonstration of *critical* temperatures. In order to carry out the experiment with carbon dioxide a tube such as shown in Fig. 7 is prepared, the wider part of which has a capacity of about 25 cc. It is clamped in a retort stand. The dried gas from a Kipp generator is led through and the (capillary) constrictions at *a* and *d* are first fused. Then *b* is wrapped in asbestos wool which has been dipped with a forceps in liquid air. When sufficient solid carbon dioxide has

FIG. 7.—Sealing in liquid carbon dioxide.

condensed in *b*, the tube is fused at *c*. The parts *a*, *b* and *c* are shown in approximately natural size.

For demonstration, the tube is clamped in a simple wire stand and put into a strong-walled test tube which is warmed while in the projection apparatus. The experiment is particularly impressive if the test tube is placed in a rocking mechanism.[3]

4. *Ethyl chloride* is, among others, very convenient for *cooling* a microsample. The author uses the "ethyl chloride bottles," little wash bottles which are sold for surgical purposes and which are closed by an easily operated valve. The stream of liquid is directed on the sample to be cooled, and temperatures as low as −20° are reached.

5. If the contents of a centrifuge cone are to be *observed under a microscope*, the lower end, containing the precipitate, is immersed in water in order to obtain clearer images. For this, the tube may be fastened to the slide by means of some wax, moistened with a drop of water and covered with a large cover glass (or piece of a thin

[3] See Appendix II. On critical solution temperatures see Z. anal. Chem. **54**, 495 (1915).

slide) as shown in Fig. 8. A small cell which may be easily fashioned from two slides and several pieces of glass cemented together with Canada balsam is more convenient.[4] The cell of course is filled with water. Small cells can also be purchased. See section on schlieren, p. 40, and Appendix II.

Of course these devices can be used only with low magnifications. If the precipitate is to be observed under higher magnifications a sample is removed from the centrifuge cone by means of a capillary tube, transferred to the slide and covered with a cover glass.

6. In general, nothing much need be said about *dishes* and *crucibles;* in any case, an adequate supply of such vessels made of transparent and dark glass (possibly of quartz) and porcelain is absolutely necessary. Their capacity should be $\frac{1}{2}$, 1, 3 and 5 cc. For covering (except crucible covers), watch glasses 20 mm. in diameter will serve. Some of these should also be made of quartz. A semi-spherical *platinum spoon* of 1-cc. capacity with a handle is very desirable for many purposes.

7. Beside the usual *wash bottles,* small wash bottles made from a

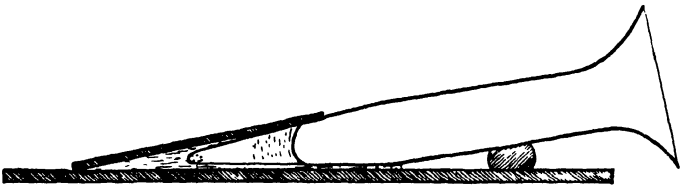


FIG. 8.—Observation of precipitate in centrifuge cone under microscope.

test tube or small flask of 25- to 50-cc. capacity should be available; they are especially serviceable in washing ("quantitative") precipitates where only a relatively small amount of liquid is used; they can be quickly heated, and, if necessary, a separate wash bottle for each kind of wash liquid can be kept at hand. If these wash bottles are fitted with glass stoppers, overlapping (cap) seals are recommended so that the reagent will not be contaminated by glass dust. (See also Fig. 54.)

8. *Slides and Cover Glasses.* As is known, two sizes of slides are in use: the "English" form, 76 by 26 mm.; and the "Cast," 48 by 28. For microchemical purposes the former is preferred, as a greater series of reactions can be carried out on a single slide. If possible, when *heating,* the entire width of the slide should be moved over the microflame so that the slide will not crack so readily. In the case of liquids which tend to creep, the slide is moved in such a way that the flame encircles the drop (Fig. 9).

[4] Illustrated, e.g., in Weigert, Opt. Methoden, Leipzig, 1927, p. 48.

A number of slides are cut lengthwise into three parts ("narrow slides") with a cutting diamond; they are recommended for experiments requiring heating. Several slides are *varnished* by covering them with a thin layer of Canada balsam-xylol mixture and placing them in a drying oven at 60 to 70° until the resin does not show the imprint of a finger nail at ordinary temperatures. These slides are used when working with solutions containing hydrogen fluoride. A pair of varnished cover glasses should also be prepared. Clear celluloid plates are more convenient than these varnished glasses. Several slides with a cavity and several with glass rings cemented on are very desirable. The latter are especially serviceable in projection experiments. The acquisition of a quartz slide (perhaps small, for instance, 10 by 25 mm.), is very desirable, and a cover glass of the same material is scarcely dispensable; however, in an extreme case one can manage with the latter alone. For most purposes, ground slides are unnecessary; suitable pieces of glass (cut, for example, from used well-cleaned photographic plates) can be used.

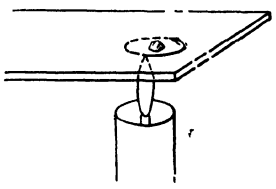


FIG. 9.—Heating creeping liquids.

III. Sundry Other Equipment

1. A small, hand-operated *laboratory centrifuge* is included among the absolutely necessary apparatus; a simple form is shown in Fig. 10. Metal shells which hang jointedly on a cross arm serve to hold larger tubes, designed, e.g., for urine examinations. The centrifuging serves to rapidly separate out a precipitate from which the clear solution ("centrifugate" corresponding to "filtrate") is drawn off.

The centrifuge cones or test tubes mentioned above can, when rolled in paper, be placed in the large sedimentation tubes. It is much more convenient to have a special attachment made for the small tubes as shown in the illustration. To prevent accidents, a cover of steel sheeting is placed over the apparatus, many firms furnishing the centrifuge with such covers. For work which requires a longer centrifuging, electrical centrifuges are very desirable.

In order to protect the centrifuge, it is important to take care that the arms carry *equal loads*. Therefore, for example, as much water is put into the empty centrifuge tube as solution to be centrifuged in the other.

In statements on centrifuges one should never neglect to consider, in addition to the number of revolutions, its *effective radius* also, as statements of the former alone are worthless.

For substitutes for centrifuges see Appendix II.

2. Among further apparatus one needs several *forceps*, including one with platinum tips ("platinum forceps"). The latter as well as the small forceps should have good gripping tips, smooth and toothless; the larger forceps, on the other hand, should be toothed. One or two *compression* forceps such as those used by surgeons to clamp blood vessels shut are very desirable.

3. Furthermore, one needs a pair of platinum wires fused into glass tubes, one of which is filed to a point ("platinum needle") the other hammered flat ("microspatula"). A fine sewing-needle clamped or

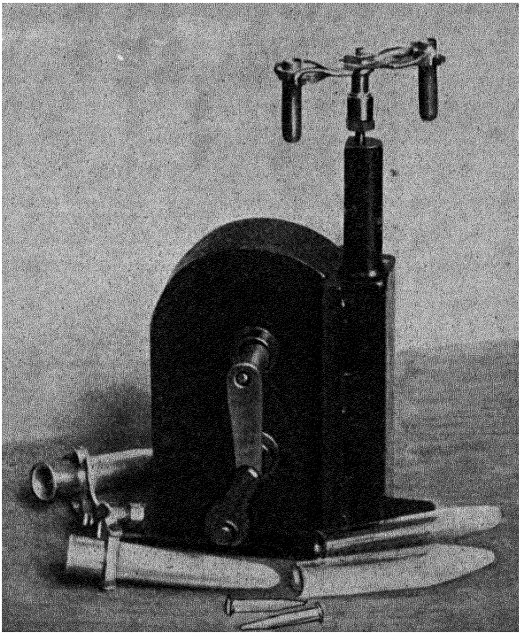


FIG. 10.—Centrifuge.

cemented with sealing-wax into a platinum-wire holder, pen holder or the like is used as preparation needle.

4. For *pulverization* of the substances the usual agate mortars ordinarily suffice; in special cases, as with materials which tend to splinter, agate mortars constructed like diamond mortars are employed.[1]

[1] Obtainable from W. Const. Wild & Co., Idar (Nahe) Germany. See Fig. 1508 in Hugershoff's price list No. 50 (1929). Experiments with chromium micromortars are planned.

IV. The Reagents, Their Preserveration, Purification and Addition of Proper Amounts

(a) Solid Reagents

Solid reagents are required especially for the Behrens microreactions.[1] It is very desirable to have a Behrens reagent box (Fig. 11) in which places are provided for vials of 1-cc. capacity with (ground-in) stoppers. The majority of the vials are made of glass; for a few especially delicate reagents, e.g., potassium-free platinic chloride or sodium-free ammonium

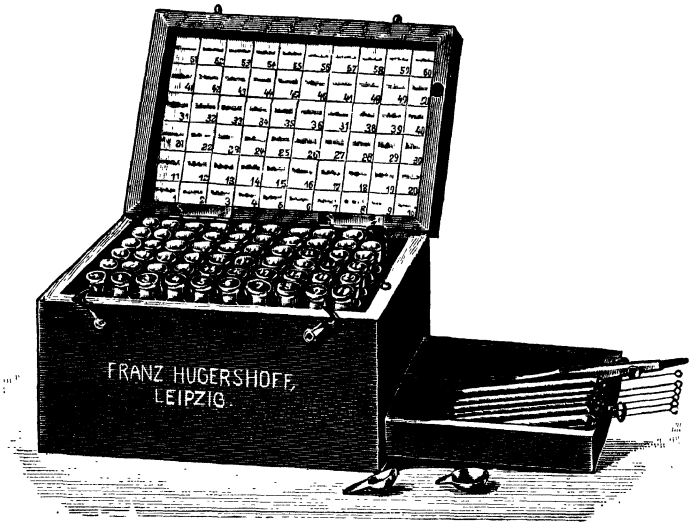


FIG. 11.—Behrens reagent box.

uranyl acetate, quartz vessels are very desirable. Hard rubber vials are also required for ammonium fluoride and ammonium fluosilicate.

In the following table, a list of the reagents used by N. Schoorl [2] is given. It includes sixty preparations. The author uses a box made to hold a larger number (say 150) of reagents, since in the course of time other needs make themselves known.

In the drawer of the box may be kept various pieces of apparatus, such as small dishes, watch glasses, glass rings, platinum spoon, spatula, forceps, platinum needle, cover glasses, slides, centrifuge cones, stirring hooks, loops, capillary tubes, filter capillaries, etc.

The reagents are placed in the vials in a powdered condition. The *removal of suitable amounts* presents no difficulties, as one quickly becomes accustomed

[1] Behrens-Kley, Mikrochemische Analyse, Leipzig and Hamburg, 1915 or 1922.
[2] Private communication.

REAGENTS IN THE BEHRENS REAGENT BOX, ACCORDING TO N. SCHOORL

Ammonium Acetate	Magnesium Acetate	Calcium Acetate	Strontium Acetate	Cobalt Acetate	Lead Acetate	Copper Acetate	Uranyl Acetate	Oxalic Acid	Gold Chloride
Calcium Carbonate	Chloro-platinic Acid	Platinum Sulfate	Cerous Nitrate	Rubidium Chloride	Caesium Chloride	Thallium Nitrate	Silver Nitrate	Mercuric Chloride	Uranyl Sodium Propionate
Ammonium Perchlorate	Ammonium Fluoride	Ammonium Silico-fluoride	Ammonium Molybdate	Ammonium Dichromate	Ammonium Cobalti-thiocyanate	Ammonium Mercuri-thiocyanate	Ammonium Bicarbonate	Di-Ammonium Phosphate	Potassium Ferricyanide
Potassium Acid Oxalate	Potassium Oxalate	Potassium Hydroxide	Potassium Carbonate	Potassium Nitrite	Potassium Chlorate	Potassium Iodate	Potassium Iodide	Potassium Chromate	Potassium Ferrocyanide
Sodium Carbonate	Sodium Peroxide	Sodium Hydroxide	Sodium Bicarbonate	Sodium Chloride	Sodium Nitro-prusside	Litmus	Turmeric	Congo Red	Potassium Bisulfate
Magnesium	Zinc	Iron	Silicon Dioxide	Starch	Gelatin	Nitron	Benzidine Hydro-chloride	Tetrachlor-quinone	Dimethyl Glyoxime

to estimating the order of magnitude (decimal place) of the quantity remaining on the spatula or platinum needle. Often it is advisable to place the heap on an empty spot on the slide and add the required amount gradually to the drop of unknown.

(b) Liquid Reagents

1. *Liquid reagents* are, as is known, more easily spoiled, i.e., they are exposed to contamination by constituents of the container material and the air of the laboratory. The author uses reagent bottles of 100-cc. capacity which are fitted with a double seal, stopper and cap. For special purposes, several quartz bottles are very desirable, and, of course, the colorless transparent material is preferable to the cloudy quartz ware, as in the case of the latter the impurities which enter the cavities during grinding can scarcely be removed. Quartz vessels are, as is known, practically impervious to water or acids, but are noticeably attacked by ammonia and considerably by alkalies. As far as the author knows, no objection has been raised to the use of tin for the storage of water.

In reference to the impurities due to the introduction of *glass dust* in bottles with *ground-in* stoppers, more is said in other books on colloid chemical investigations. Glass dust can also be a disturbing factor in microanalysis; for example, it may form a sediment in the point of the "capillary" (pp. 27 f.) and give the impression of a precipitate. In order to make sure, the reagent is always taken from *inside the bottle* and not from the drop hanging from the stopper. Of course, unnecessary grinding while opening or closing the reagent bottle is to be avoided. Likewise, when one and the same reaction is to be repeated, a large drop is placed on the slide and small droplets are taken from this as needed.

Often glass containers can be protected by simply paraffining them.

Reagents which deteriorate in air are enclosed in "reagent-capillaries" described by F. L. Hahn.[3] These are 1 to 2 mm. in diameter and 5 to 6 cm. in length, drawn out at the end to a point 0.1 to 0.2 mm. in diameter, and are sealed at both ends after being filled with the liquid. When using, one end is broken off and the liquid allowed to flow out, e.g., on the slide by heating with the microflame.

2. For purposes of practice and comparison, solutions of all the common ions are used the concentration of which is approximately known. It seems to the author to be immaterial whether 1 per cent or 0.1 normal solutions are used. The author prefers the former, because one can determine the weight of the ion in question from the size of the drop by simple mental calculation and *because the limit of identification*

[3] Mikrochem., Emich-Festschrift, p. 143 (1930).

of microreactions has, up to now, always been given in micrograms (see p. 2; occasionally micromilligram, μ mg. or $m\gamma$, is also used).

3. *For the purification of reagents by distillation*, W. Lenz uses an apparatus consisting of a Jena glass distilling flask of 1.5-liter capacity with a neck 15 cm. long, and a quartz condenser tube. For the preparation of *pure water*, 1 liter of water is treated with 10 g. of potassium bisulphate and 1 g. of potassium permanganate, allowed to stand for 12 hours and then distilled. The first 100 cc. serve to wash out the condenser, which has already been well cleaned. The next 300 cc. are collected (in a quartz vessel). The *condenser tube* is 50 cm. long, at least 6 mm. wide and with walls about 0.75 mm. thick. The angle at which it is bent is 60 to 70°. The shorter shank with the obliquely ground end projects at least 1.5 cm. below the sealing cork. Of course, a ground joint is better than a cork. The condenser shell is 25 cm. long. Smaller flasks are used for the distillation of acids, fractionation flasks of quartz being the most convenient.

As a rule, it is sufficient to have a quantity of *purest water* at hand, and the other volatile reagents are purified *immediately before use.* This can be done most simply by first dipping a (quartz) glass rod in pure water and then saturating the drop which remains on it with the gas in question by holding in the hydrochloric acid, ammonia, etc., reagent bottle above the liquid. The saturated drop is then tipped off on the slide.

The reagents purified in this way always leave a microscopic residue on evaporation on the usual slides, owing to the solubility of the glass; on quartz slides the residue is usually barely visible directly, but is easily visible with dark-field illumination.

It is obvious that, for particular purposes, special precautionary measures are necessary. For halogen determinations, Pregl[4] tests the *water* by treating 10 cc. with 5 drops of nitric acid and an equal amount of silver nitrate solution and heating for 10 minutes in the boiling water bath. The test must not show any opalescence. In order to exclude impurities, the same author closes the mouth of the stock bottle with a soda-lime tube. He distils *nitric acid* with the addition of silver nitrate and, of course, either under vacuum or by leading in a stream of carbon dioxide which has been washed in a sodium carbonate solution. Pregl uses a bottle of brown glass as stock bottle for the acid.

4. *Addition of the proper quantity of liquid reagents requires the greatest care.* One should remember each time *how much was taken in the macroreaction and proceed accordingly.* As one often works with droplets of several cubic millimeters, the usual drop from a wash bottle or bottle represents a twentyfold excess, i.e., *an amount which is almost always excessive and which in many cases may hinder the positive result of a reaction.*

[4] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 127.

In order to minimize such mistakes (most common with beginners) the amount of reagent, as when working on a larger scale, is either (*a*) measured off approximately or (*b*) added in very small amounts until a certain result is attained.

(*a*) Either capillary tubes or platinum loops are used for measuring liquids.

(*α*) The *capillary pipets* for exact work can be made from thermometer tubing. They are calibrated with water (delivery!) and divided, e.g., 10 mg. into ten parts. The tube should be about 15 cm. long and have a circular bore of $\frac{1}{3}$-mm. diameter.

Also several measuring pipets of perhaps 2-cc. capacity divided in 0.01 or 0.02 cc. are very desirable.

A convenient form of pipet is shown in Fig. 12. The tube contains 0.02, 0.05, 0.1, or 0.2 cc. from the mouth to the mark *M* (button of black glass or the like). The values are marked on the wide portion.

Capillary tubes drawn out over the burner suffice for those cases in which the tube is used first for the test drop, the spoiled end then broken off (and thrown away) and finally the same tube is used for measuring the reagent. One can then readily determine whether the latter occupies as much space as the test drop or much more, etc.

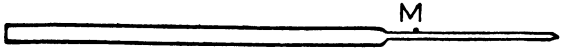


FIG. 12.—Pipet of e.g. 0.05 cc. capacity.

For the measuring apparatus of F. L. Hahn see the original paper.[5]

(*β*) The *loops* are made of platinum wire bent in a circle and soldered with a bit of gold so that the ring is closed.[6] About three loops are used, the largest of which holds 4 mg. of water, the second 1 mg. and the smallest 0.2 mg. For the larger loops, 0.4-mm. wire will serve; for the smaller, wire half as heavy.[7] The (inner) diameters of the loops are 3, 1.5, 0.7 mm. respectively. The capacity is controlled by weighing, by drawing the contents into a capillary. Of course the loop is fused into a light handle. For this purpose a thin-walled glass tube will serve. A little piece of paper on which the capacity of the loop is marked is pushed into the open end.

The *cleaning* of the loops is carried out as a rule by dipping consecutively in hydrochloric acid 1: 1, running water, and distilled water where they are left until used. (Of course, the hydrochloric acid is to be replaced by another solvent, depending upon the particular case.)

[5] Mikrochem., Pregl-Festschrift, p. 134 (1929).

[6] A tiny kernel of pure gold is placed at the point to be soldered and the wire heated in the Bunsen flame.

[7] A so-called "micrometer gauge," obtainable at hardware dealers, is used for measuring the thickness of wire (and sheet metal).

The loop is ignited directly before use. Cleaning acid and distilled water are kept in vials; the former can be used for a long time, the latter, naturally, must be renewed at short intervals.

It will be noticed, in the use of the loops, that the drops are equal in size only if they are removed from the solution in the same way. One should accustom oneself to dip the loop *in the stock bottle* and not withdraw it too rapidly. (When the loop is withdrawn very rapidly from the reagent a larger drop will remain on the hook.) The loop is then tipped off on the slide until it is empty; the droplets, which are close to each other, are merged. The procedure is similar when working with the centrifuge cone.

(*b*) In order to add very small amounts of reagent little by little to the test drop, the somewhat modified Streng "hook" (Fig. 13) is used. This is easily prepared by bending one end of a platinum wire 0.3 mm. in diameter at a sharp angle. The bent-over part is about 1 mm. long. A glass rod 1.5 mm. thick is fused on the other end for a handle. If the stirring hook is dipped in a solution and not withdrawn too rapidly, a droplet of about 0.1 mm.[3] remains in the point of the angle. It is then introduced into the test solution, and by twirling between forefinger and thumb the reagent and test drop are thoroughly mixed. The amount of liquid remaining in the hook is fairly constant; it is even possible to carry out rough titration experiments.

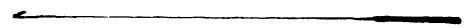


FIG. 13.—Stirring hook.

In order to save platinum, it is possible in many cases to use *glass threads* (possibly with a ball on the end), which are prepared beforehand in large numbers like the capillaries and discarded after use.

(c) Gaseous Reagents

1. Since as a rule only a very weak stream of gas is necessary for our purposes, the familiar set-up (Fig. 14), shown in one-quarter natural size, can, for reasons of economy, be used in place of the large Kipp apparatus.

Usually it will be found that *one* such apparatus, which contains ferrous sulfide on the right and hydrochloric acid of 1.1 specific gravity on the left, will suffice. The stopcock is connected with a wash bottle filled with moistened pieces of porcelain. When the apparatus is not in use, the acid bulb is lowered. If a drop of solution in a centrifuging cone is to be saturated with the gas, a glass capillary of about 0.1-mm. diameter is used from which the gas bubbles stream in the form of a fine string of beads. (A wider tube which forms larger bubbles will throw some of the contents out of the centrifuge cone.)[8]

[8] For other simple gas evolution apparatus see W. J. Allardyce, J. Chem. Education **5**, 49 (1928), or L. W. Winkler, Z. angew. Chem. **31**, 1, 64 (1918).

Very often one can apply gaseous reagents by laying the slide with the test drop underneath over the mouth of the bottle containing the solution of the gas concerned, concentrated hydrochloric acid, bromine water, ammonia, ammonium sulfide, etc.

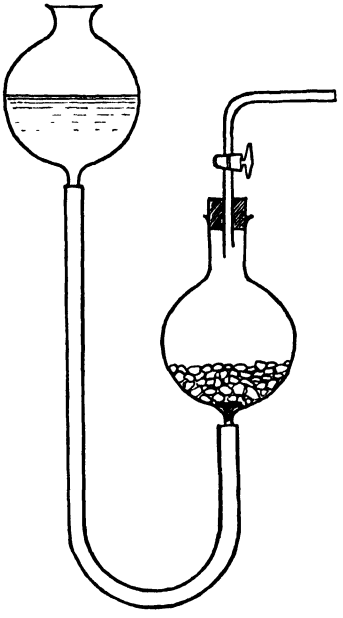


FIG. 14.—Gas evolution apparatus.

2. *The Gas-chambers.*[9] A glass ring *R*, 15 to 20 mm. wide and 5 to 15 mm. high, which is ground even on its upper and lower edges, is placed on a slide I (Fig. 15); a slide II (or a cover glass) closes the chamber. The reagents which evolve the desired gas are placed on the bottom, and the drop which is to be treated with it hangs from the cover of the chamber.

The gas chamber also serves as a microdesiccator for qualitative purposes. A piece of calcium chloride the size of a lentil can be used as drying medium.

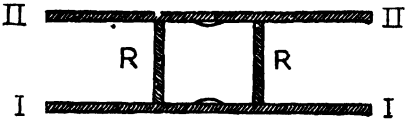


FIG. 15.—Gas chamber.

Another simple gas chamber consists of a test tube (centrifuge cone) closed with a cork. The test drop is held in a platinum (glass) loop which is stuck in the cork. In order to prevent spattering from one droplet into the other, a plug of cotton can be placed midway between the drops. In the case of the gas chamber, a piece of filter paper will serve the same purpose.

In the same way, of course, the chamber can be used when a test is to be kept moist for a long time.

V. Treatment of Precipitates

The separation of precipitate and solution can be carried out in very diverse ways; there are especially a large number of microfiltration methods. The following procedures may be mentioned:

[9] Molisch, Mikrochemie der Pflanze 65, Jena, 1921; Emich, Lehrbuch d. Mikrochemie, Munich, 1926, p. 41. Mayrhofer uses for the same purpose a sort of double chamber. A. Mayrhofer, Mikrochemie der Arzneimittel und Gifte, I, 7.

(a) Treatment of the Precipitate on the Slide

1. When it is desired to separate the precipitate and solution rapidly, *drawing off* is recommended. It is effected by drawing the solution away from the precipitate by means of a platinum needle or glass thread by inclining the slide slightly and drawing the clear part of the drop little by little to one side. The drawn-off parts are collected into a larger drop by a sort of rotary motion, and the liquid is thereby separated as completely as possible from the precipitate. At the same time the slide is inclined more and more. Whether a precipitate is fitted for this procedure can be seen as a rule by introducing the needle into the liquid: if the precipitate spreads to all parts of the drawn-out drop the drawing-off procedure cannot be used, but if the precipitate remains quietly in place, the method can be used with success. It can be used especially with heavy cheesy precipitates (calomel), with those which adhere to the glass (metallic mercury) or those which become very flocculent (ferric hydroxide). Behrens recommends that the solution containing the precipitate be evaporated and then extracted with the solvent to facilitate the drawing-off.

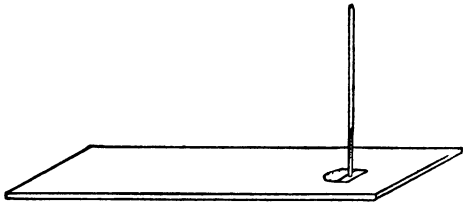


FIG. 16.—Filtration according to Hemmes.

2. A very convenient filtering procedure is due to Hemmes. A rectangular or trapezoidal piece of loose, thick filter paper, at the most 8 to 10 mm. long, is placed (Fig. 16) beside the drop to be filtered. The *evenly ground*[1] end of a little pipet is touched to the paper and suction is applied at the upper end by means of a rubber tube leading to the mouth. It is to be noticed that the pipet is to be only *lightly* pressed on the paper and that the lower end sticks closely to the paper. With very small amounts of liquid and with narrow tubes, sucking with the mouth is unnecessary, capillary action sufficing.

Nothing much need be said about washing the precipitate; as a rule a few drops of wash liquid will suffice. If the filtrate is not to be used one can often accomplish the same end by repeatedly touching a strip of paper to the moist precipitate. Another drop of water is then placed on the precipitate, etc.

3. A procedure which is equally simple and often useful consists of closing an end of a capillary tube with a clean cotton or asbestos plug and dipping it into the turbid solution; the clear filtrate rises in the tube

[1] N. Schoorl, private communication.

and can be worked with further here, or blown out after removal of the plug. In the case of some precipitates the plug may be dispensed with.

(b) Sedimentation and Decantation in Centrifuge Cones

Sedimentation and decantation in centrifuge tubes is as a rule the cleanest and simplest procedure for the separation of a precipitate. With very heavy precipitates, silver chloride, for example, which is coagulated by heating and stirring (stirring hook), standing for a short time will suffice for the precipitate to settle out. Usually the same result is attained by brief centrifuging, and then it only remains for the clear solution to be drawn off by means of a finely drawn-out pipet (diameter 0.2 mm.) or a capillary siphon.

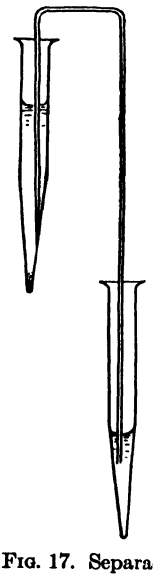


FIG. 17. Separation of precipitate and solution by means of a capillary syphon.

(*a*) In the first case, the capillary pipet is dipped farther and farther into the drop until directly over the precipitate (see also Fig. 19), and the centrifuge cone and pipet inclined more and more until almost horizontal. Often the centrifuge cone can actually be inverted without any of the precipitate entering the pipet. When the precipitate is separated as completely as possible from the solution, the latter is blown out into a second centrifuge cone, wash liquid is added to the precipitate, stirred with the stirring hook or glass thread (p. 23), centrifuged again, etc.

(*b*) In the second case, i.e., the application of the capillary siphon, the centrifuge cone is taken in one hand and in the other a capillary of about 0.75-mm. bore which has been bent into a ⅂-form; the right branch of the capillary is in the (empty) centrifuge cone in which the solution is to be collected. As soon as the siphon is dipped into the solution, the liquid begins to flow over to the second centrifuge cone. If necessary the centrifuge cone is inclined. If the left (shorter) branch of the siphon is drawn out to about 0.2-mm. bore it will remain filled even after removal from the solution and can be used again, i.e., until the washing is completed. While the centrifuge cone (left) is being centrifuged, the right-hand cone in which the wash water is collected is placed together with the siphon in the block, Fig. 4, p. 12. See also Fig. 17.

If the precipitate is to be dried, the set-up shown in Fig. 5, p. 13, is used.

With very small amounts of liquid it is not expedient to change too

often the vessels with which one works; this applies particularly to work with capillaries, which will now be discussed.

(c) Treatment of Precipitate in Capillary Tubes

The necessary tubes of 1 to 2 mm. inner diameter are usually drawn from well-cleaned tubing and kept ready for use in lengths of 30 to 40 cm. In general they are used only once, as cleaning requires too much time. In certain cases quartz capillaries may be necessary. The following manipulations are carried out most frequently.

1. If a solution in one capillary I (Fig. 18) is to be transferred to another capillary II this can be accomplished with a single turn of the crank of the centrifuge if the tubes are placed in the centrifuge in the position shown in the illustration.

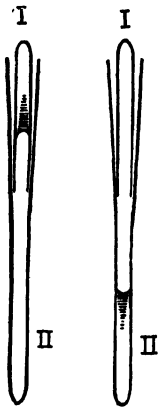


FIG. 18.—Transfer of solution from I to II.

2. If the solution over a precipitate in tube I (Fig. 19) is to be drawn off, an improvised pipet II is employed which as a rule will fill perfectly with the clear solution when the system is sufficiently inclined. Sometimes, to be sure, it happens that traces of the precipitate reach II. This can be overcome by sealing II at *a* over a microburner, centrifuging the solution toward the end *a*, then sealing at *b* and driving the precipitate into the point. The end *a* is then opened by taking off the cap, and finally the tip *b* is cut off above the precipitate and the solution is clear. We will assume further that it is still to be treated with a reagent. For this, the end *b* is dipped into a drop on a slide while *a* is closed with the finger. By lifting the finger the necessary amount is allowed to enter. If the liquid requires mixing, both ends are sealed again and the tube centrifuged several times, each time reversing its position in the centrifuge. (It need hardly be said that the tubes can be cleaned, if necessary, at the places to be sealed by centrifuging a droplet of water down to the solution.) All these manipulations take but little time.

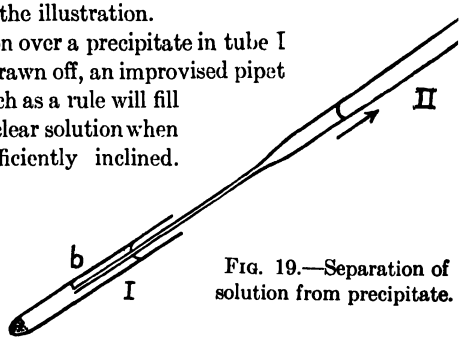


FIG. 19.—Separation of solution from precipitate.

3. We will assume further that a precipitate has formed when the solutions were mixed. It is forced into the point by centrifuging and examined under the microscope with low magnification. Sometimes it is expedient not to mix the solutions too rapidly but rather let them diffuse into one another. It is then possible to obtain readily recognizable microcrystals in the capillary which can be observed in the cell described on p. 15. Of course, these capillaries can be kept as evidence when sealed.

4. It is obvious without further comment that the precipitates may also be

washed according to the described procedure and their behavior toward various solvents tested.

5. If the test is to be *heated*, the procedure given on p. 13 is followed with the (sealed) tube.

6. If a precipitate does not settle out completely because some particles stay obstinately on the surface of the liquid, generally the familiar expedient of adding alcohol will help.

7. F. L. Hahn[2] uses capillaries of 0.3 to 0.4-mm. width and about 15-cm. length. They are drawn out as in Fig. 20 and broken apart at the point ↓. The reagent is allowed to enter through the point (e.g., 5 mg. oxyanthraquinone in 2 cc. *N* sodium hydroxide for the detection of magnesium which we shall use as example), the point is sealed, the upper part of the tube is allowed to bend by its own weight when heated in the microburner, and the tube is centrifuged briefly. If an air bubble is seen in the narrow part on examination under the microscope, it can be easily removed by moving the capillary over the microburner from *A* toward *B*. It is again centrifuged until any precipitate present in the reagent is completely collected in *A* and appears reddish. It is then broken at the constriction between *A* and *B*, the test solution allowed to enter and the tube sealed and again centrifuged. After 5 minutes at the most the tube may be examined under the microscope. If a group of tubes are to be tested at the same time, the open end, for example, is sealed, bent or the like in different ways. See also Exercise 74.

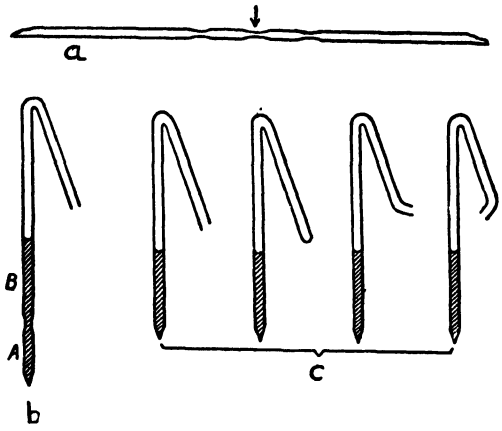


FIG. 20.—Hahn capillaries.

a, Center of capillary before breaking apart; *b*, Capillary with reagent; *c*, Capillaries with reagent and various solutions, identified by various shaped ends. By sealing the open ends of the two last ones, two new forms are obtained. (Length approximately natural size. Width magnified 4 to 5 times.)

(d) Other Methods of Filtration

Of the numerous other filtering methods, we may mention that of Strzyzowski.[3] He uses little glass funnels, which are made in five sizes.

[2] Mikrochem., Pregl-Festschrift, p. 137 (1929).

[3] Osterr. Chem.-Ztg., 123 (1913). Obtainable from F. Hugershoff, Leipzig, Carolinenstrasse.

The funnels (Fig. 21) are constricted somewhat at *A*; above this constriction they are filled with purified asbestos with the aid of a wire. The height of the asbestos layer is about 1 to 3 mm.; the plug must not be pressed too tightly into the funnel opening. The asbestos is thoroughly washed with hydrochloric acid and water, for which the centrifuging mentioned below will serve as well as the usual suction apparatus. After that, the funnel is placed in a test tube or centrifuge cone, as shown in Fig. 22, filled with the liquid to be filtered, and centrifuged, first slowly and then gradually faster to the maximum speed. Generally a clear filtrate is obtained from the first filtration, but it may, of course, be filtered a second time. A pipet, drawn out hair-fine, is used in working further with the filtrate. Liquids of 5 cu. mm. volume can be filtered; in such a case, only about 10 per cent of the amount poured on is retained by the filter.

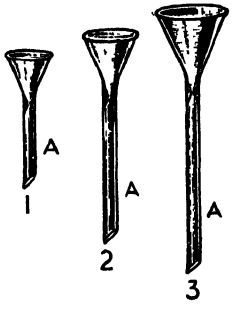


FIG. 21.—Strzyzowski funnels.

FIG. 22.—Funnel and catch vessel in large centrifuge cone.
W, water.
F, filtrate.

The funnels can easily be made by allowing a capillary tube to collapse in the microburner. The manner of manipulation can be seen from Fig. 23, the microburner being used for heating. In certain cases it is advisable to seal the funnel at *a*. The lower end of the funnel is then cut off after washing the precipitate and the further procedure is as described under "capillary tubes." Very small drops are placed in the funnel in a small piece of tube (opening of course downwards) before centrifuging.

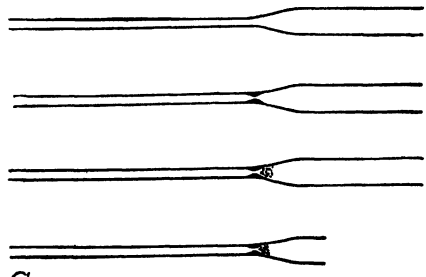


FIG. 23. Preparation of Strzyzowski funnels.

VI. Recrystallization

1. *For preparative purposes* Pregl[1] uses the "microbeaker," i.e., the 4- to 5-cm. end of an ordinary test tube of 14 mm. diameter, the rim of which is bent out and formed to a lip at one point (Fig. 24). In dis-

[1] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 222.

solving the substance, one twirls in the liquid a glass rod 1 mm. thick and 12 cm. long, the end of which is fused to an obliquely hanging drop. The hot solution must not be completely saturated. It is filtered through cotton or asbestos in a microfunnel (see illustration) which is drawn out from a test tube. The spherical widening in which the filter material is only lightly pressed is 5 mm. in diameter. The filtered solution is allowed to cool with stirring and filtered by means of the Schwinger micro suction filter[2] (Fig. 25).

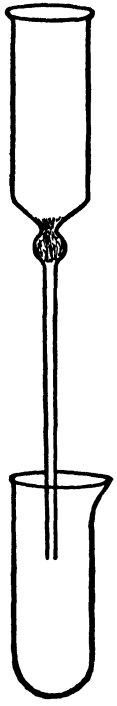


FIG. 24.—Microfunnel and microbeaker of F. Pregl. (Natural size.)

This consists of a thick-walled glass tube of 10 mm. outer diameter and 2- to 2.5-mm. bore. The upper end is ground off flat and polished, the lower cut off obliquely. The upper part of another tube of the same outer diameter but of 3- to 3.5-mm. inside diameter is widened into a cylinder 35 mm. long and 10 mm. wide. The bottom end of this upper tube fits exactly to the tube that was ground flat, and it is also ground flat and polished. These two glass pieces are connected by a piece of rubber tubing, after a microfilter (circular piece of hardened filter paper[3] cut out by a cork-borer) has been placed between the two ground surfaces. The usual apparatus can be used for applying suction, e.g., a small bell jar (p. 76) connected to a pump.

FIG. 25.—Schwinger micro suction filter. (½ natural size.)

After filtration, the rest of the crystals are washed out of the beaker with a little wash bottle into the funnel, washed if necessary and finally pressed out, after which the rubber tube is pushed over the lower tube and the crystals removed from the upper by a glass rod.

2. The Donau filters work very conveniently and cleanly (p. 75).

3. With easily soluble substances it is often better to carry out the filtration *in the microbeaker itself*. This can be done in the following way. A tiny plug of asbestos is placed in a tube of 1 mm. bore (Fig. 26) and is fused into the end as shown (enlarged three times) in the side diagram. This can be done quickly by heating the end of the tube for a short time by turning in the microburner. The tube is bent as shown in the illustration and connected with a suction apparatus, the side tube of which is connected to a pump. As the crystals are pushed

[2] See also Haushofer, Mikroskop. Reaktionen, Braunschweig, 1885, p. 160.
[3] Monatsh. Chem. **30**, 745 (1909).

together with the end containing the asbestos, the mother liquor is collected in the container *C*. In order to do this, the microbeaker is held in the left hand and the container *C* in the right. Finally a strip of hardened filter paper is placed in the crystal mass, the beaker is sealed with a cork and allowed to stand for several hours. In place of the hardened filter paper, a small quantity of (cleaned) thread will serve very well. In place of the suction apparatus (Fig. 26), a glass capillary drawn out to a point at the end will often suffice; it may be placed in an almost horizontal position in the crystal pulp or it may be helped by sucking with the mouth. The contents are transferred by blowing out into a microbeaker. The introduction of the above-mentioned strip can rarely be avoided. After repeated filtration, the asbestos filter occasionally stops up. Some pure solvent is then drawn through.

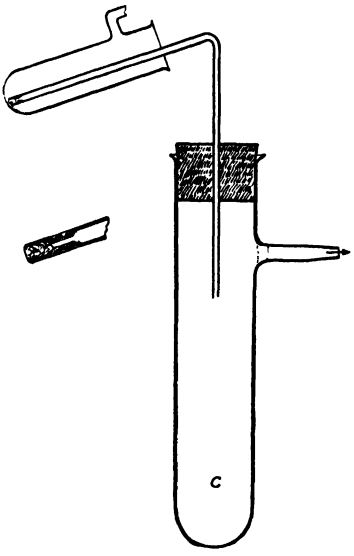


FIG. 26.—Filtration of crystals in microbeaker.

4. For recrystallization on the slide see the specific part.

5. *Recrystallization in sealed capillaries* is distinguished by its particular simplicity and presents the possibility of great economy of material. The reader is first referred to page 127 where a procedure with acetanilide is described. A method of working is described which, e.g., can be used in conjunction with schlieren observations of *easily melting substances.*[4] A capillary 7 to 8 cm. long (Fig. 27) is used, which has an asbestos plug (not shown) at the constricted point.

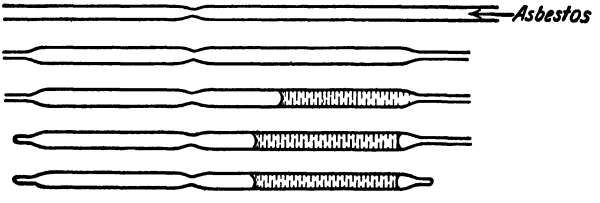


FIG. 27.—Recrystallization in sealed capillaries.

The liquid (e.g., 30—50 mm^3.) to be examined is drawn into one end which has been drawn out into a point, the other end is sealed. The

[4] Worked out by H. Häusler, Monatsh. Chem. **53/54** 312 (1929). The description of the method of *fractional* crystallization is also in this paper.

asbestos plug must not be wetted. After the sealed end has cooled, a partial vacuum is formed which causes the liquid to be drawn in and away from the open point so that this may also be sealed.[5] The outer diameter of the capillary may be from 1 to 3 mm., depending upon the amount of liquid. The portion of the tube filled with liquid is brought to the proper temperature in a cooling mixture. When the mixture congeals, the tube is centrifuged in a centrifuge cone (Fig. 28). The mother liquor goes through the plug, the crystals pack together at the constricted portion of the tube over the asbestos plug. The capillary is then cut off at *a* (Fig. 28), the mother liquor (eutectic) is sucked up in a capillary pipet and can be used further as desired. The crystals (at *K*) which have thawed in the meantime, can be treated in an analogous manner, after the second part of the tube has been cut off at *b*. If there is a possibility of the entire crystal mass thawing during the centrifuging, one can proceed as shown at the right in Fig. 28.

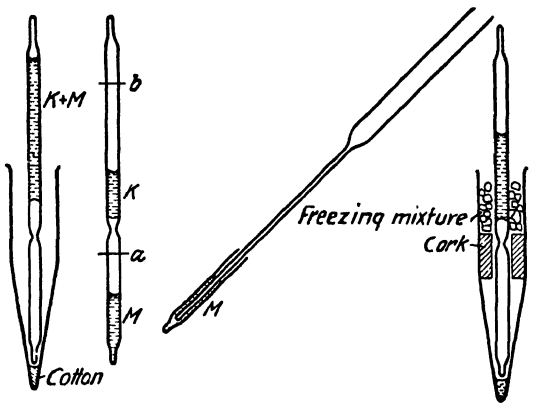


FIG. 28.—Recrystallization in sealed capillaries.

6. For working with the "micro centrifugal filter" of Pregl, the reader is referred to his book.[6]

VII. Boiling-Point Determination and Fractionation

1. Boiling-Point Determination [1]

A number of tubes (I, Fig. 29) are made from well-cleaned soft glass tubing (Pyrex glass cannot be used) by repeated drawing out.

[5] In sealing care must be taken that, owing to *decomposition*, no appreciable amounts of impurities enter the sample.

[6] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 224.

[1] The methods of melting-point determinations are assumed to be familiar. See also H. Meyer, Analyse u. Konstitutions-ermittlung, Berlin, 1922, p. 100; also for melting-point determination under the microscope, G. Klein, Mikrochemie, Pregl-Festschrift 192 (1929); and Chamot and Mason, Handbook of Chemical Microscopy, Vol. I, p. 300.

The tubes are 7 to 10 cm. long and have an outer diameter of 0.6 to 1.2 mm. and a wall thickness of about 0.01 mm. The tubes are open at both ends, one of which is drawn out to a very fine point about 2 cm. long (see below). If this is dipped into a drop of the liquid to be examined (which may also be in a melting-point tube if the boiling-point determination is to follow a determination of the melting-point) the liquid will rise slowly and the required amount of about 1 cu. mm. will fill the narrowed (conical) part. The end with the capillary point is then sealed by drawing out or by merely touching with a small flame. By sealing in this way, a little gas bubble is formed in the point of the capillary. The "*boiling tube*" filled in this way is shown in II.

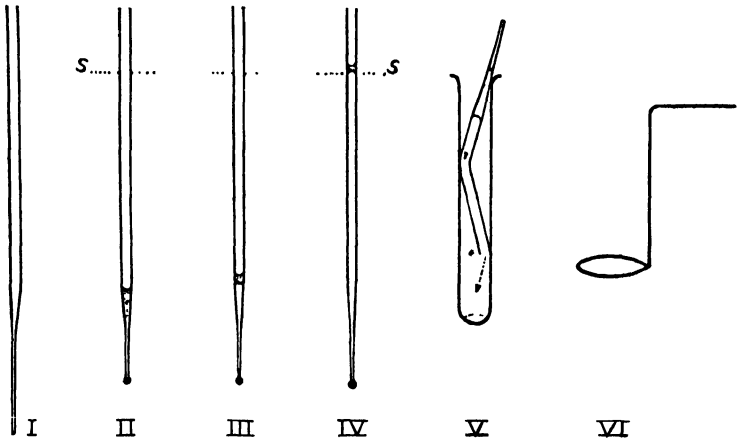


FIG. 29.—Boiling point determination in capillary tube.

It is to be noted that the success of the experiment depends upon the presence of this bubble. It is, however, also essential that the volume of the gas bubble be vanishingly small compared to the volume of the bubble of vapor which is formed later. For the dimensions already given and for a width of 0.05 to 0.1 mm. of the capillary point, a bubble length of about 1 mm. has proved satisfactory. Of course, it may be also shorter and therefore wider.

If the bubble happens to be too big, it is sometimes possible to reduce it by centrifuging the tube whereby part of the gas is removed. Usually, however, in such cases it disappears completely and it is simpler to fill a new tube. In order to keep the loss of substance to a minimum, the spoiled tube is cut off and slightly bent. It is then placed in a small test tube as in V, Fig. 29, and the droplet centrifuged into it; it may then be drawn up into a tube again, etc.

The size ratios given are approximate; they can be measured with the mag-

nifier by laying the tube on a half-millimeter scale etched on glass.[2] As soon as the required practice has been obtained, the measurement may, of course, be dispensed with.

The prepared boiling tube is fastened to a thermometer like the melting-point tube, e.g., with saliva, and placed in the bath which is filled with the heating liquid to a height of at least 4 to 5 cm. A glass tube (rod) of the familiar form (VI) serves as a stirrer. Heating may be rapid at first, but as soon as the bubble enlarges (III) and the drop begins to become restless, the heating is slow and the bath stirred vigorously. The drop rises and finally reaches the meniscus *SS*, and *with that the boiling-point is reached.* It is often possible to make a series of readings from one and the same tube by allowing the drop to cool and fall and by reheating cause it to rise again. Now and then, of course, it happens that, when this is done, a second drop will form higher up and enclose a large bubble. In this case further observation cannot be made. With easily mobile liquids, e.g., ethyl ether, it has occasionally happened with the author that the drop did not rise as a continuous column, but a real boiling was then observed.[3]

2. Fractionation

First Procedure: The maximum number of fractions is desired, the boiling-points of which are to be determined.

The "fractionation tube," Fig. 30, is a glass tube 50 to 60 mm. long and with an outside diameter of 5 to 8 mm. It is sealed at one end and drawn out into a short handle. A wire can be fastened (by winding around) to the handle and will serve for holding the tube while heating. A narrow brass tube about 12 cm. long is more convenient.

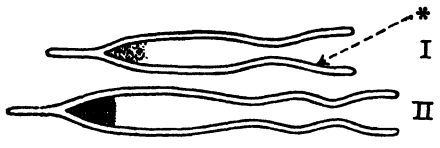


FIG. 30.—Fractionation tubes.

The glass tube has a constriction in the middle and can easily be prepared by drawing out an ordinary soft glass or Pyrex tube. (For low-boiling substances a tube (Fig. 30, II) with two constrictions is

[2] Such glass rulers are the size of a slide and can be obtained from optical firms.

[3] A *melting-point determination* may be combined with the boiling-point determination, but in this case it is necessary to fill the boiling tube with the *molten* substance (and not with the powder which would form too large gas bubbles). In order to seal the capillary tip in such a case, the tube end must be heated above the melting-point during the sealing, which can be accomplished most simply by laying the tube on a heated metal sheet. As Chr. J. Hansen notes, the procedure is also applicable for reduced pressure.—Houben, Methoden der organischen Chemie, I, p. 850 (1925).

used and a moist piece of linen is wrapped around the part of the tube between the constrictions.) Some asbestos, previously purified by boiling with concentrated hydrochloric acid, washing with distilled water and finally igniting, is placed at the bottom of these fractionation tubes. After use the tube can be freed of all volatile impurities by weak ignition.

The boiling-point capillaries (Fig. 29) are used to draw up the individual fractions and determine their boiling-points. A beaker

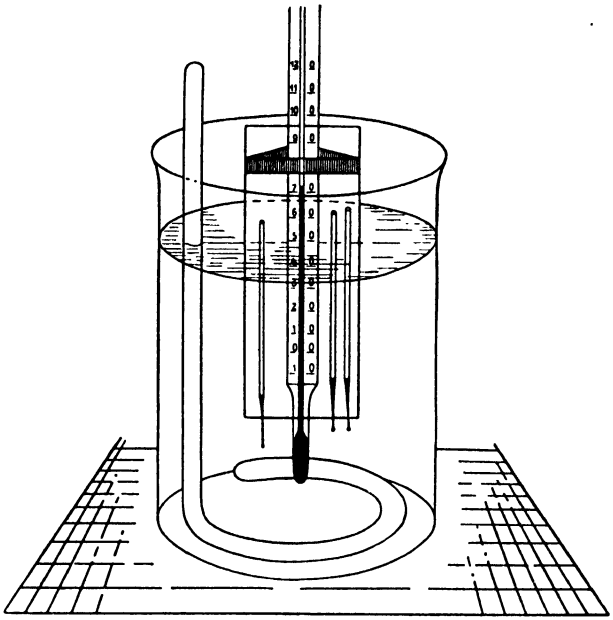


FIG. 31.—Determination of boiling point of small amounts of liquids.

filled with sulfuric acid or paraffin oil serves as heating bath (Fig. 31). A thermometer to which a slide is fastened by a rubber band is dipped into the beaker. The boiling-point capillaries with the individual fractions adhere to this slide. As many as ten may be held at one time. *They are arranged in the order in which they were filled.* By this arrangement it is possible to determine the boiling-points of all the fractions at once. A simple stirrer of glass serves to maintain homogeneous distribution of heat throughout the bath liquid.

If the boiling-points of *high-boiling* liquids are to be determined, droplets of the liquid may condense in the part of the boiling-point capillary projecting from the bath before the boiling-point is reached. It is advisable in such a case to use a round flask with a wide neck closed by a cork with a hole for the thermom-

eter and a notch in the side. Furthermore, a boiling-point capillary which is not too narrow is selected, and it is immersed as far as possible in the heating bath.

The method of working is as follows: 1 to 3 drops (=0.05 – 0.2 g.) of the liquid mixture is introduced into the fractionation tube. In order to get the liquid as completely as possible in the end of the fractionation tube containing the asbestos, the tube is placed in the centrifuge and the drops forced to this end by several turns of the crank. In order to remove the last portions of the liquid mixture from the upper part of the fractionation tube, which is particularly necessary with high-boiling substances, the open end of the tube is drawn through the flame several times and then allowed to cool. For fractionation the tube is slowly heated over the microburner with the lower end about 5 cm. above the flame. This must be done very carefully and with continuous rotation of the tube. One observes that a little boiling ring appears which moves past the constriction of the tube. At this moment the heating is stopped and the tube laid almost horizontal. The distillate collects in the form of a droplet at the point marked * in Fig. 30, I. The droplet is then drawn up into a boiling-point capillary. The remainder of the distillate is centrifuged back into the tube and the procedure repeated to the last fractionation. The series of boiling-point determinations is then carried out in the apparatus shown in Fig. 31. It is also possible to submit the first and final fractions to a further separation, as in the usual method of fractional distillation with large amounts of liquid. If it is desired for this purpose to transfer the fractions back into the fractionation tube from the boiling-point capillaries, the tube is centrifuged with the capillaries (open and downward) whereby the fractions are brought back on the asbestos.

Second Procedure:[4] *Only one or a few fractions are desired from a very dilute solution as for example in the detection of traces.*

This method employs the same principle as used in the foregoing procedure and is described in detail by Benedetti-Pichler and Schneider.[5]

The distilling flask (Fig. 32) was made of Pyrex glass in various sizes. In the illustration the dimensions of the smallest flask (capacity 12 cc.) are given. Flasks for 60-cc. and 120-cc. capacity have almost the same dimensions, with the exception of the part *a*.

Before use the apparatus is well cleaned and freed from grease with "permanganate-sulfuric acid"[6] and then rinsed out well with water.

[4] Inserted by translator.

[5] Z. anal. Chem. **86**, 69 (1931).

[6] A few crystals of potassium permanganate are dissolved in about 5 cc. concentrated sulfuric acid in a dry test tube. The reader is reminded of the dangerous character of this solution and is cautioned to use extreme care.

The tube from the bulb up is dried by stroking with the Bunsen flame. It is well to clamp the apparatus in a stand in the position shown in the figure. When drying, it is turned 180° on its longitudinal axis. This position is also better for filling than the one shown in which the distillation is carried out. It is necessary to dry the tube in order to facilitate the introduction of the material used to prevent bumping. For this purpose zinc dust or 20-mesh powder has been found satisfactory. In certain cases sodium bicarbonate is used in place of the zinc on account of the reducing action of the latter. The bicarbonate is also added in the solid form (about 1 g.).

The test solution must be introduced slowly so that it does not fill the tube and thus prevent the air within the flask from coming out. Zinc dust or sodium bicarbonate which may have adhered to the walls of the tube is washed down into the bulb by the test solution.

The narrow tube is then again dried by playing the flame of a Bunsen burner on it. After cooling, the actual distillation is begun. The solution is heated by an easily regulated microburner which is fitted with a chimney to protect it against air currents. As soon as the liquid begins to boil, the flame is regulated so that the formation of vapor takes place as slowly as possible. A condensate ring which appears in the tube should rise so slowly that it requires 1 to 2 minutes to reach the knee *c*. As soon as the condensate ring enters the descending part *cd* of the knee section, the flame is lowered so that no more distillate enters the knee-formed part of the tube. The boiling, however, must not be interrupted at this point, as otherwise, owing to the condensation of the vapor filling the tube, air will be drawn through the tube which may result in drawing part (in the case of very volatile liquids, all) of the condensate back into the flask. This action could be plainly seen in experiments with ethyl ether solutions.

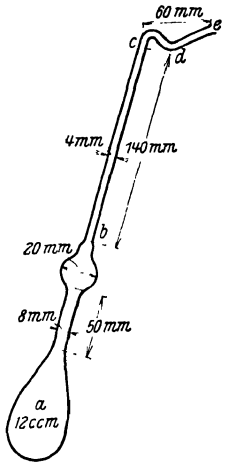


FIG. 32.—Fractionation flask of Benedetti-Pichler and Schneider.

As soon as the condensate ring collects at *d*, it is taken up in a boiling capillary or capillary pipet. Its boiling-point may then be determined as described above. Solid carbon dioxide ("dry ice") is used as cooling medium in the case of very volatile substances such as ether or chloroform. The dry ice is crushed in a mortar and piled around the knee part in a small aluminum trough which is clamped on the same stand. Of course, carbon dioxide snow may also be used. In either

case, the cooling medium evaporates without leaving a residue, thus permitting clean working.

In experiments with ether solutions it was noted that, when a large steam bubble in the bulb burst, the ether vapor was driven upwards or, if it already filled the knee part of the tube, was driven out into the air. This led of course to loss of material. Placing a tight-fitting glass plug with a capillary bore in the mouth of the tube prevented this loss.

In the case of very soluble substances such as methyl and ethyl alcohol it was possible to obtain distillates of high concentration in one distillation. With substances which are only slightly soluble or not

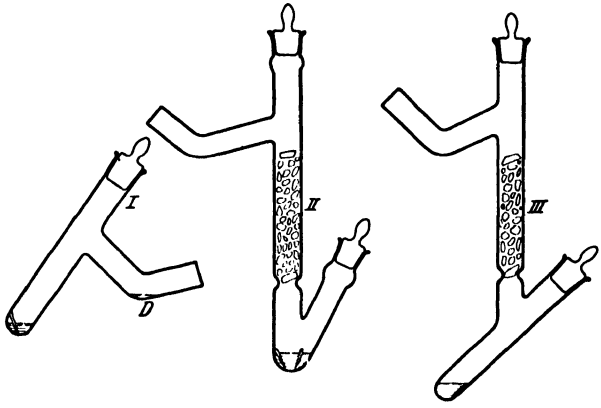


FIG. 33.—Fractionation flasks.

miscible in all proportions such as ether and chloroform, the distillate consisted of the pure constituent. The limit concentrations with some of the substances used in the experiments described are ethyl alcohol 5 : 100,000; acetone 5 : 100,000; ether 1 : 10,000. In all these distillations 60 cc. of the original liquid were used.

Third Procedure: Only one or a few fractions are desired: e.g., in the purification of a liquid; which perhaps are to be compared with one another.[7]

The distillation is carried out in apparatus as shown in Fig. 33. The use of a column filled with pieces of porcelain is often expedient although not necessary in all cases.

For working with the little apparatus I about 0.1 cc. of the liquid to be examined is necessary; for the larger apparatus II and III with columns, about 0.1 to 0.3 cc. Handling small amounts of liquid is

[7] Monatsh. Chem. **53, 54**, 312 (1929).

simple if suitable pipets are used. Several boiling capillaries, i.e., glass tubes of 1 mm. outside diameter and 1 cm. length, sealed at the top and drawn out fine at the bottom, are introduced in the boiling sample. The horseshoe-shaped boiling capillaries of A. P. Knoebel[8] are also excellent. The distillate collects at *D* and is removed with a pipet, to be used, e.g., in a schlieren experiment (p. 40).[9]

VIII. Sublimation

The great interest in micro sublimation methods is shown partly by the fact that there are a considerable number of procedures. Several methods for (*a*) qualitative analytical and (*b*) for more preparative purposes are presented here.[1] The requirements of technical work and botany are not considered.

(a) Methods for Qualitative Analytical Purposes

1. *Sublimation from Slide to Slide.* The substance is placed at the end of a narrow slide which is held in one hand. A second slide is held ready in the other. The first is heated by means of the microflame and, as soon as volatilization begins, is removed from the flame. The second slide is then held over the first so that condensation takes place on the former. The procedure requires a certain amount of practice, and loss may easily occur not only because of the escape of vapor but also because the slide may (not infrequently) crack. On the other hand, the advantages are the rapidity and the possibility of carrying out further microreactions with the sublimate in the most convenient manner.

It is obvious that a mica plate or platinum spoon may be used in place of the first slide when required, as for example with difficultly volatile substances. Very small porcelain crucibles are occasionally convenient. A platinum crucible cover cooled by a drop of water may also serve as receiver.

2. Kempf recommends that the sublimation be carried out so that the vapor need travel through only a very short distance, e.g., a few tenths of a millimeter, and also that the sublimation proceed at the lowest possible temperature. In order to obtain a sublimate despite

[8] Chemist-Analyst **19**, No. 1, 20 (1930).

[9] Recently we have introduced a thermometer in apparatus I.

[1] See also Emich, Lehrbuch d. Mikrochemie, Munich, 1926, pp. 56 f. Of other publications the following may be mentioned: A. Mayrhofer, Mikrochemie d. Arzneimittel u. Gifte, Vienna and Berlin, 1923, I, 16; 1928, II, 10; G. Klein, Praktikum d. Histochemie, Vienna, 1929; Wagenaar, Z. anal. Chem. **79**, 44 (1929); Chamot and Mason, Handbook of Chemical Microscopy, Vol. I, p. 348.

this, the substance is heated for *hours* and possibly under vacuum. In its simplest (and for our purposes usually sufficient) form the procedure consists of placing the slide with the substance on a flat metal block Fig. 72 (p. 129), and using a cover glass for collecting the sublimate. One or two glass threads between the cover glass and slide set the height of the sublimation chamber.

3. *The methods described in the next section under* (*b*) 2, *work conveniently and economically,* but the dimensions of the sublimation tube may be reduced to about 2 mm. inner diameter. Various apparatus use special cooling devices (cited on p. 39, footnote 1).

(b) Preparative Methods

1. In many cases the old procedure of subliming between watch glasses will suffice.

2. Pregl[2] places the substance in a glass tube 20 cm. long and 7 mm. outer diameter. It is laid in the regenerating block (Fig. 47, p. 64) and there heated to a definite temperature. The tube is closed at one end and open at the projecting end if sublimation is to be carried out at ordinary pressure. If it is desired to employ vacuum the open end is connected to a pump. Of course a stream of inert gas, again either under ordinary or reduced pressure, can be used. When the sublimation is completed, the tube is removed and cut, and the sublimate is pushed out by a sharp-edged glass rod.

IX. Schlieren Observations [1]

(*a*) *General.* A. Toepler defines *schlieren* as regions of changing refraction in an otherwise optically homogeneous medium. The schlieren phenomenon has been utilized since Toepler's time to make visible phenomena in ballistic, physical and physico-chemical experiments. We compare two liquids, one, the "static sample," in a little cell (Fig. 34) which for most purposes can be made from slides and pieces of glass cemented together. (The firm of Zeiss of Jena and New York furnishes cells made entirely of glass fused together.) The other

[2] Pregl-Fyleman: Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 226.

[1] Ostwald's Klassiker 157 and 158. F. Emich, On Observation of Schlieren in Chemical Work, Monatsh. Chem. **50**, 269 (1928) and **53/54**, 312 (1929). Since work has been carried out in this field for only the past few years, only the most important points will be presented here. All details as well as other applications, e.g., the measurement of schlieren strength, etc., can be found in the original work. This section is taken in part from a lecture of Benedetti-Pichler, Z. angew. Chem. **42**, 954 (1929).

liquid, the "fluid sample" is allowed to flow into the static sample from a capillary which is drawn out at the bottom to a bore of about 0.15 mm. and which is 1 to 2 mm. wide at the upper part. In order to be able to start the flow conveniently, the fluid and static samples are separated by drawing an air bubble into the outlet tip of the capillary before dipping into the static sample. The pressure of the fingers on the rubber tube drives out the air bubble and the fluid sample begins to flow in.

(*b*) The *observation* of schlieren can be carried out in many different ways. Only two methods are briefly presented here: working with the schlieren microscope and the "visual" method.

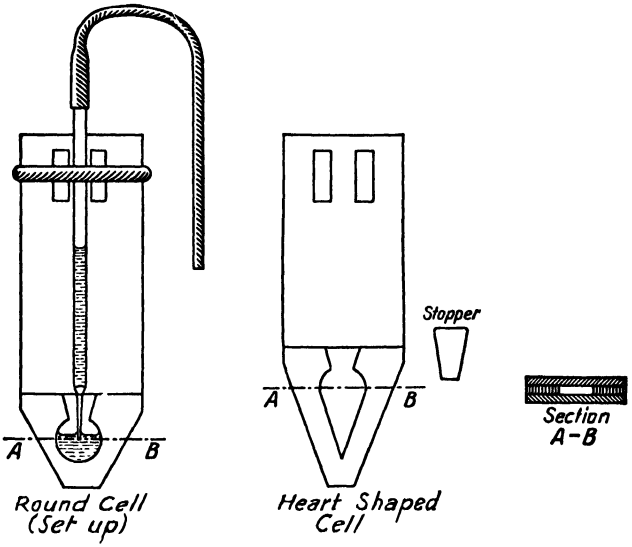


FIG. 34.—Cells for schlieren observation.

The schlieren microscope is shown in Fig. 35. It consists of the illumination slit *B*, the table *H* which can be rotated about the optical axis and on which the cell is clamped in a vertical position, and the horizontal microscope which can be rotated about the vertical axis *A*. In the tube of the microscope is a second slit, the "tube slit." The illumination is supplied by a frosted electric lamp which is placed back of and near the illumination slit. The two slits, which are placed parallel to each other, are arranged so that the optical inhomogeneities of the portrayed object stand out distinctly. (See also the section on the determination of refractive index, p. 11.)

The field of the schlieren microscope shows three zones (Figs. 36 and 37) when the tube has been set up for the observation of the schlieren: one (left) side shadow with hazy edge, a brightly illuminated central field and a right-hand shadow with a more distinct edge. The schlieren are most clearly visible when their image appears at the edge of the hazy shadow.

If the shadow of the schlieren is adjacent to the hazy shadow, the fluid sample has the *higher* refraction: the schlieren are designated as *positive*. If the fluid sample is less refractive than the static sample, the schlieren shadow will be opposite the hazy shadow: the schlieren are termed *negative*.

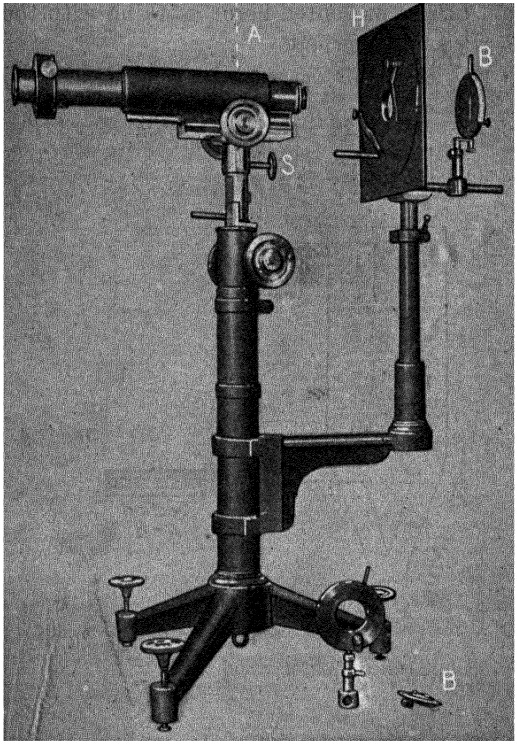


FIG. 35.—Schlieren microscope.
(Explanation of letters in text)

But the schlieren phenomenon shows not only small differences in refraction but also, and in a highly sensitive way, the difference in *specific gravity* of the two liquid samples. Depending on whether the fluid sample has a higher or lower specific gravity than the static sample, the schlieren are designated as "descending" or "ascending."

(*c*) In the analytical application of schlieren phenomena, the determination of concentration is next in importance. The solution to be tested is allowed to flow into different static samples of known concentrations. In the transition from the nearest comparison solution of higher concentration to the next lower comparison solution, the schlieren undergo a double change. For example, the negative, ascending schlieren of the former test change to positive and descending schlieren.

The schlieren method is particularly valuable for *testing purity*,

especially in the determination of the uniformity of substances isolated for the *first time.*

In the examination of *liquids* the following courses are open:

1. Comparison with a standard preparation.

2. The sample is subjected to fractional distillation and the distillate is compared with the residue ("phlegma") or the distillate or residue allowed to flow into the original sample. The appearance of schlieren, if the possibility of decomposition at the boiling temperature is excluded, proves that the liquid is a mixture and that at least a partial separation of the components has taken place during the distillation.

3. The separation of the mixture by fractional melting according to p. 31 is tried; the mother liquor *M* is transferred by the capillary pipet into a cell (Fig. 34, p. 41). The thawed crystal mass is drawn into a

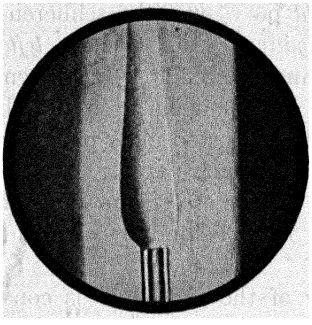


FIG. 36.—Descending positive schlieren.

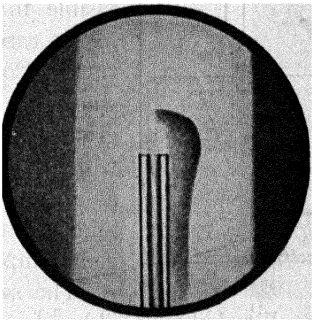


FIG. 37.—Ascending negative schlieren.

capillary and the two liquids are allowed to flow together. The appearance of schlieren shows the presence of impurities.

(*d*) Furthermore, the schlieren method can be used among others for the *detection of ferments.* For example, a small piece of a wafer is fixed (with Canada balsam) in a cell filled with water. Even after long standing no material changes are noticeable on the wafer. If a trace of saliva is added to the water by means of a platinum loop, the ptyalin begins after a time to hydrolyze the *starch.* Descending positive schlieren appear dropping from the wafer.

Or a *flake of fibrin* is placed in a cell with 0.1 *N* hydrochloric acid. If the fibrin has been previously soaked in acid of the same concentration, it will show no further change. Ten minutes after the addition of a solution of a pepsin preparation, the decomposition of the protein becomes noticeable, as strongly positive schlieren sink to the bottom from the fibrin flake.

(e) *Schlieren Observation with Unaided Eye.*[2] Schlieren can (as shown by everyday experience) be observed with the unaided eye. This so-called "visual method" has the great advantage of extreme simplicity so that it can be employed in working with large and small amounts of material especially for purposes of testing purity, without the need for special apparatus. The fluid sample is allowed to flow out of a capillary into the static sample which is contained in a cell. The cell is held in the distinct range of vision so *that one can see the (straight) line of separation of a dark-bright field* (window cross-bar, house edge) *directly beside the capillary* (Fig. 38). Assuming that the *dark* part of the field lies to the *left* of the dividing line, the shadow in the case of *positive* schlieren will be visible in the *right* portion of the schlieren and in the case of a *negative* schlieren in the *left* portion. Positive (and descending) schlieren are shown in Fig. 38. A particularly suitable background is formed by a frosted glass plate, half of which is covered with black paper. It is placed at a distance of ½ to 1 meter from the observer and is illuminated from behind by an incandescent lamp.

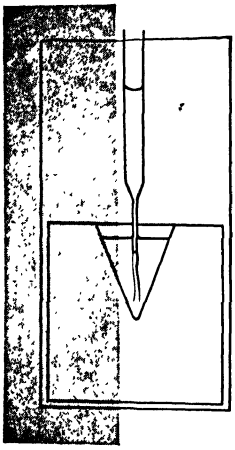


FIG. 38.—Visual schlieren observation. Dark portion of "field" at left.

The sensitivity of the procedure is considerable when the schlieren are observed at the hazy separation edge; *a difference in index of refraction* [Δn_D] of 0.0001 *may be determined by the sign* (whether + or −) *and direction of flow* (whether ↓ or ↑) as soon as the eye is somewhat accustomed to the method of observation.

Test tubes (diameter 17 to 19 mm.) may also be used for the static sample in place of the cells with plane parallel sides if on the one hand sufficient liquid is available and on the other hand no particularly great sensitivity is demanded. A Δn_D of 0.0005 is also possible to determine by sign; schlieren in general already become visible at Δn_D of 0.0003 to 0.0004. The relation: shadow of schlieren to line of separation is directly opposite to that observed in cells, since in the round test tube the dark portion of the background appears on the opposite side.

X. Qualitative Microelectrolysis [1]

A method for applying electrolysis to qualitative analysis which could also be used in trace detection has recently been described.[2] In

[2] H. Alber and Maria von Renzenberg: Z. anal. Chem. **86**, 114 (1931).

[1] Inserted by translator.

[2] H. J. Brenneis, Mikrochemie, Kongressheft, 385 (1931).

principle, the method consists of reducing the electrode surface to a minimum so that, despite the fact that only very small amounts are present, the metals can readily be seen on examination of the electrode under the microscope.

The Rod Electrode. Platinum or platinum-iridium wires (*A* and *B* in Fig. 39) of suitable dimensions are fused in a thick-walled glass capillary *K* so that the wires are insulated from one another by glass but are only about 0.1 mm. apart. The capillary is 5 mm. in outer diameter and 0.5 mm. bore. It is cut at the point of fusion, ground flat and polished, whereby the platinum wires appear as tiny plates imbedded in the polished surface of the glass. The ends of the wires projecting from the lower end of the capillary are soldered to flexible lead wires. This electrode is fastened in a fitting glass tube *G* with picein, thus forming a rod which is less fragile and more easily handled than the electrode itself.

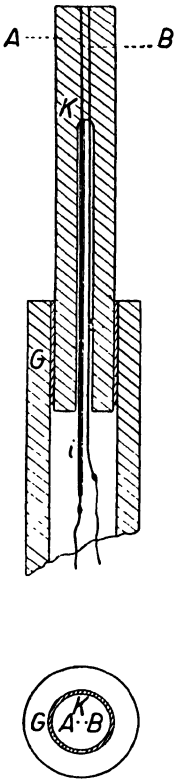


FIG. 39.—Rod electrode.

The Needle Electrode. In carrying out the electrolysis in vessels such as crucibles, platinum dishes, etc., a needle electrode is best. It is the same as the rod electrode only a thinner capillary is used. The outer diameter of the latter should not exceed 1 mm.

The Electrolytic Slide. By fusing three wires instead of two in the capillary, a three-pole electrode is formed. This three-pole electrode is used in the form of an electrolytic slide which is employed in carrying out electrolyses of drops whereby evaporation of the drop during extended electrolysis is prevented. One advantage of the third electrode lies in the fact that, in a simultaneous anode and cathode deposition, it will serve as a comparison surface since it is not connected with a source of current. Fig. 40 shows cross-section and top view of such an electrode. The electrode is fixed with picein in a plate of hard rubber the size of a slide. The three poles are connected to the binding posts *A*, *B*, and *C*. A hard rubber ring *R* serves for holding the cover glass and can be raised or lowered by turning on the thread of the mounting of the electrode. The wires of 0.1-, 0.05- and 0.025-mm. diameter have been used.

Working with the Electrodes. The method of working is practically the same for all metals. The solution to be examined, of course prop-

erly acidified, etc., is placed on the electrode in the form of a small droplet (1 to 0.1 cu. mm.) by means of a calibrated platinum loop or glass capillary (p. 22). The electrode is connected in the usual way with a battery, resistance and voltmeter. The metal deposits on the cathode (or anode) and is washed carefully without interrupting the current by placing a droplet of water on the electrode surface by means of a fine capillary, removing it with another capillary or a piece of filter paper, and repeating as often as necessary. The deposit is examined under the ordinary microscope using a vertical illuminator or under a binocular microscope with good table lamp placed beside the instrument. In the latter case it is often advisable to incline the electrode somewhat. The electrolysis requires a few seconds or hours, depending

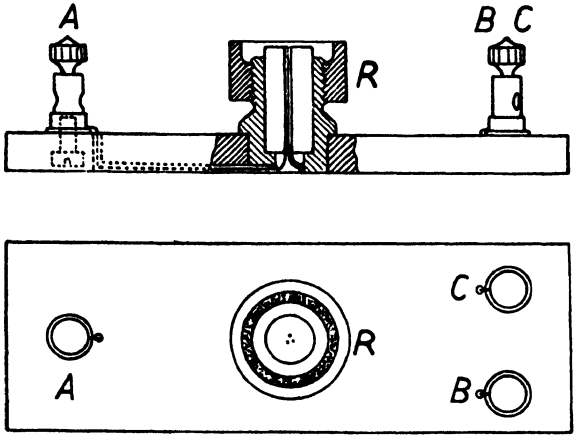


FIG. 40.—Electrolytic slide.

upon the amount and the electrochemical behavior of the metal. When the process requires hours, evaporation of the electrolyte must be prevented. In the case of liquids containing sulfuric acid, the hygroscopic character of the latter suffices to keep the solution from evaporating. With other electrolytes a moist chamber consisting of a test tube lined with moist filter paper is placed over the rod electrode, and hinders evaporation. In the electrolytic slide, the same effect is obtained by placing a wet cover glass on the ring *R* and turning the latter down so that the cover glass touches the test drop.

If for any reason the deposition of the ion in question takes place any better on another metal than platinum, the platinum electrodes may be previously covered with the proper metal by electrolysis.

In some cases the color or form of the deposited metal or compound (such as the anode deposits of PbO_2 or MnO_2) suffices for the recognition of the ion; in other cases special reactions must be carried out for the identification. For example, silver may be identified by the formation of silver sulfide by exposing the deposit to the fumes of ammonium sulfide or touching a drop of the reagent to it. With larger amounts of the metal (0.01 γAg) it is possible to apply the silver dichromate reaction (red coloration).

For further details, especially as to the preparation of the electrodes and special tests to be applied to the deposited ions, the reader is referred to the original paper.

XI. Mounting Permanent Microchemical Preparations

Even though a freshly prepared comparison preparation is preferred to all others, in certain cases one would like to have permanent preparations at hand. They can be very desirable, e.g., in legal-chemical investigation. A little collection is also useful for projection and other instruction purposes. However, less emphasis should be placed on show pieces obtained by employing all possible

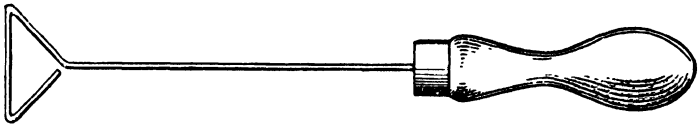


FIG. 41.—Wire tool for sealing permanent preparations.

tricks, than upon preparations which show *those* forms and *those* arrangements which are met with in analysis.

1. In many cases it is sufficient simply to protect the test drop from evaporation, which is possible in various ways. For example, it can be covered with a cover glass, the excess displaced liquid removed with filter paper as completely as possible and the edge sealed with vaseline. For this purpose a wire of the form shown in Fig. 41, which is dipped while hot into the sealing medium, is used. Preparations which are to be photographed can easily be kept in this way for the required time.

2. *Crystalline precipitates*, if they are to be preserved in a dry state, must as a rule be first separated from the mother liquor and washed. It is hardly possible to give directions which would govern every substance. Often it is sufficient to draw off the mother liquor with a pointed strip of filter paper. In this case, care must be taken to contaminate the preparation as little as possible with fibers. Hardened filter paper meets this requirement well, but has less absorbing action than the usual thick filter paper or suction board, which is preferred in the case of viscous mother liquors. A drop of wash liquid is then placed on the crystal mass and is again drawn off and so on. Whether water or 50 per cent

alcohol or some other liquid is to be used for washing depends upon the particular case; often it is advisable to wash once with water and then once or twice with dilute alcohol. After the last drawing off, the crystals are allowed to dry and then brought under a preparation lens or binocular microscope. The impurities are then removed by means of a needle, the preparation perhaps again carefully examined under the microscope and finally sealed with a cover glass and a paper ring.

3. *Balsam Preparations.* Preparations which are to be sealed in balsam are first treated as described above in 2, i.e., washed and dried. The imbedding itself is effected by first placing a drop of benzol or chloroform on the dried object so that the preparation is as free from bubbles as possible. A drop of balsam is added, and then, if necessary, heated for several hours (in the drying oven) at 70 to 80° so that the resin hardens. Preparations which cannot be heated are kept in a place protected from dust (bell jar) for 24 to 48 hours before sealing. Finally a drop of balsam is placed on a cover glass and this is allowed to drop on the preparation.

It is important that the index of refraction of colorless objects be not too close to that of the imbedding medium. Therefore crystals which are nearly invisible in Canada balsam ($n = 1.54$) are imbedded in metastyrol. Behrens also recommends a solution of dammar resin ($n = 1.50$). All these resins can be used in benzol solution, Canada balsam without further treatment. The solution of dammar resin may be prepared by extraction (reflux condenser) and filtration of the extract.

The sealed preparation may be further provided with a *lacquer ring.* For this purpose it is placed on a turntable, centered, a little brush is dipped in black "mask lacquer" and ring made with one stroke if possible. By application of the lacquer ring an absolutely dust-tight seal is always obtained with dry preparations, and with balsam preparations it ensures the adherence of the cover glass. Besides, the preparations have an attractive appearance. Finally they are cleaned and labeled.

B. Quantitative Section

I. Introduction. Historical Notes, Limitation of the Field

If one understands by quantitative microanalysis the determination of quantities of material which cannot be carried out with the required accuracy of 0.1 to 0.2 per cent with the usual analytical balance, the beginnings of the field extend quite far back. We may mention what the author believes to be the oldest procedure—the measurement of metallic spheres under the microscope (Vict. Goldschmidt, 1877).[1] This

[1] See also the chronological presentation in Benedetti-Pichler's reference work on the progress of microchemistry in the years 1915–1926.—G. Klein and R. Strebinger, Fortschritte der Mikrochemie, Vienna, 1927, pp. 131–436. (At this point a typographical error in this book may be corrected: on p. 133, seventh line from the bottom should be "589" and not "289".)

method has recently gained in significance by the work of Fritz Haber and his students. (Still older are several colorometric and nephelometric methods, but these lie essentially outside the field of this book.) The work of Nernst (1903), Krogh (1907) and myself (from 1909) and my coworkers is of later date. Among the latter I must particularly mention Pilch and Donau, who among others had already determined nitrogen, halogens and sulfur in organic compounds. After the feasibility of working with several milligrams of analytical material had been demonstrated by these investigations, the first publication of Pregl on quantitative organic microanalysis appeared in 1912 and the second in 1917. Since about 1920 the amount of work in this field has increased enormously.

The limitation of the field of "quantitative microanalysis" is unscientific in so far as it does not set any lower limit. It would be better, for example, for the present to give the decimal place (order of magnitude) of the quantities of end substance. In this respect the designations proposed by the author "centigram" and "milligram methods"[2] (identical with semi-micro and ordinary micromethods) already give more information. In an analogous manner, work with thousandth milligrams would be designated as "gamma methods"; "decagamma methods," when the next higher decimal place comes into consideration, etc. But perhaps even still better one can say 10^{-2} or 10^{-3} method (whereby, as in *p*H values, further abbreviations will develop later). So, e.g., "10^{-4} method" is a method in which we work with 0.000 *n* g. of substance.

Often in the introduction of a nomenclature in a field in the process of development, too little thought is given to its possible future development. The result is that names are created which, such as the combinations with micro, ultra-micro, semi-micro, etc., signify nothing objective, but instead, in the most favorable cases, conform only to the present state of experimental knowledge. One should avoid them as far as possible and replace them with those which express *numerically* what one desires to say. For example, an "ultra-micro balance" appeared recently which was said to (apparently[3]) permit weighing to 0.1γ with a load of 10 to 20 g. This name was, in the first place, unsuitable, as its inventor naturally could not know what improvements the future would bring, and in the second place, it was incorrect as there were already more sensitive balances. Every such uncertainty would be impossible if the balances were designated not by terms such as "micro" or "ultra" but by their actual performance. One could, e.g., name a balance which permitted weighing 10 g. with an accuracy of 1γ a "gamma-decagram balance." This would be simple and definite and would not anticipate in any way. Our usual analytical balance would then be a "milli-hectogram balance." I do not suggest that just these words must be chosen, but the general idea seems to me to be worthy of consideration. (The combinations are by no means as monstrous as those which our

[2] Ber. dtsch. chem. Ges. **43**, 29 (1910).

[3] Perhaps exact directions for its use are lacking.

conceptions of compound structure compel us to use, even if one must use more exact statements as, for example, two deci-milli-five decagram balance, etc.). A certain confusion also arises from the fact that "micro" is used in two different senses, namely (qualitative) as small, e.g., in "microscope" and "microbalance" and (quantitative) in the sense of 10^{-6}, e.g., in "microgram" and "microfarad."[4]

II. Balances, Weighing and Quantitative Micromethods in General

1. *Introductory Remarks.* There are a large number of "microbalances."[1] In this connection it must first be said that fine physical balances sensitive to about 0.001 mg. have been known for a long time. Most of them were vacuum balances which, intended, e.g., for standardization purposes, were not convenient enough for usual laboratory work. Later the *Nernst balance* especially proved very satisfactory in hundreds of determinations. Unfortunately it can carry a load of only 50 to 100 mg. Credit must be given to Pregl for discovering in the assay balance constructed by Kuhlmann (which the author had already been using since 1906) an instrument which, with expert handling, permitted weighing not to about 0.01 mg. as originally thought, but even to 0.001 or 0.002 mg. And since it permitted a load on both sides of 20 g., it, above all, cleared the way for the carrying out of organic microanalyses, in which greater demands are made on the load capacity of the balance chiefly because of the absorption apparatus. But the introduction of the microbalance constitutes an advance also in *inorganic* work as it is now possible to use crucibles, filter tubes, etc.[2]

In general, in a quantitative determination only *one* property is measured; most often the weight or the volume. Occasionally, however, other properties, which it is assumed bear a simple relation to the mass, are measured, such as color, refractive index, rotation of the plane of polarized light, etc. The weight is usually not determined directly, but, e.g., only the *increase in weight* of a filter tube in a series of manipulations. It is assumed in this case that, first, the tube itself does not change in weight and, secondly, that the increase in weight is

[4] See, e.g., Küster-Thiel, Rechentafeln, Berlin and Leipzig, 1929, p. 102.

[1] See also my presentation in Abderhalden's Handbook, 9th Supplement, pp. 55–147, given again in an abbreviated form in Abt. I, T. 3, of the same work, pp. 183 ff. (Vienna and Berlin, 1921).

[2] In the third edition of his Quantitative Organic Microanalysis (Philadelphia, 1930), Pregl contradicts (p. 8) a statement made by me in the Lehrbuch d. Mikrochemie (Munich, 1926) that the Kuhlmann balance No. 19b had essentially the same load capacity as the later, improved "microchemical balance." I will have more to say about this difference in opinion at another opportunity; according to a letter which I received from Professor Pregl in the fall of 1929, it may be a matter of misunderstanding.

due only, e.g., to pure barium sulfate. Neither assumption is wholly justified in reality, as there are no absolutely weight-constant receptacles and no absolutely pure precipitates. A series of conditions always influence a complex system, and it is therefore necessary to regulate these conditions in such a way that only a certain reaction (which may be called the principal reaction) is carried out more or less completely and all other reactions of the system are forced to the background so that for all practical purposes they do not come into consideration.

2. *Classification of Methods.* Because of the large number of micromethods, it is hardly possible to consider them collectively from one common viewpoint. If they are to be placed in groups, the following can perhaps be distinguished. The amount of initial material (sample) is, of course, assumed to be known.

A. The determination form is weighed:
   (*a*) as (ignited) residue (1),
   (*b*) as electrolytic precipitate (2),
   (*c*) as (insoluble) precipitate (3),
   (*d*) as increase in weight of an absorption apparatus (4).

B. The determination form is measured (volumetrically):
   (*a*) as gas bubble or column (5),
   (*b*) as metallic sphere, crystal or the like (6),
   (*c*) in the form of a titrated solution in which the numerous possibilities of determining the end point provide considerable variation (7),
   (*d*) as column of precipitate (8).

C. The quantity of determination form is determined by optical means based on certain changes of property:
   (*a*) color intensity, fluorescence . . . (9),
   (*b*) light intensity of a spark, flame (10),
   (*c*) intensity of refraction, polarization . . . (11).

In this list, the indirect methods of gravimetric analysis and much else are omitted, especially the field of molecular-weight determination. For our purposes, cases 1, 2, 3, 4, 5, 7, and 11 come into consideration as particularly important practical methods, but here also a simple consideration shows that the individual differences of the particular cases render the general treatment difficult. We will try to select the most important practical viewpoints.

3. The greatest simplicity in procedure is shown by the *residue determinations* if they are practically free from errors of method (vola-

tility of substance and the like). If the determination form (e.g., a noble metal) is identical with the sought-for constituent, the determination errors are given by the weighing errors, which can be reduced to 1 to 2γ with the Kuhlmann balance. The sample of about 5 mg. is more than sufficient to reduce the analysis error, e.g., in the case of a 50 per cent residue, to about 0.1 per cent.

4. There is unfortunately no uniformity in statements of the *error* of analysis. It is best expressed in "per cent of the result," as it is obvious that a difference of 0.1 per cent in result between found and actual value signifies *something entirely different* when one finds 99.9 per cent instead of 100.0 per cent, 9.9 per cent instead of 10.0 per cent and 0.9 per cent instead of 1.0 per cent. Although the *absolute* error is "0.1 per cent" every time, in the first case $\frac{1}{1000}$, in the second $\frac{1}{100}$, in the third $\frac{1}{10}$ too little will be found. The *relative* error is 0.1, 1, and 10 per cent, respectively. Although some authors[3] have already remarked about these (quite self-evident) relations, they are always again left out of consideration.

It is, of course, not correct in this connection to adhere to the principle that just two decimal places should always be given in the per cent constituent. The second decimal may just as well be affected by a great uncertainty as with a small, in fact, under certain circumstances, with no uncertainty. It would be much better to give, as proposed, e.g., by Saar (cited above[3]) in any case only three places, e.g., 0.376, 3.76 or 37.6 per cent.

In general, a moment's thought will show whether the last place is certain or not. If it is not, the "rounding off" of the preceding place appears to me to be more correct than the addition of a figure which may probably be wrong. At least one should make an uncertain place known to others, e.g., by the notation 37.6_4 per cent, or the like.

These considerations are, of course, correspondingly applicable to all other cases.

5. If, for example, a residue determination is carried out using the Kuhlmann balance with but 0.3 mg. of substance, the attainment of a relative precision greater than about 1 per cent is a matter of chance. On the other hand, it is easily possible to carry out residue determinations, e.g., by means of the electromagnetic balance, using 0.02 mg. of sample, and despite this an accuracy of 0.2 per cent can be expected.

It is proportionately propitious if the relative precision remains the same within wide limits with diminishing masses. This appears to

[3] See also, e.g., Saar, Z. Unt. Nahr.-u. Gen.-M. **47**, 169 (1924); Thiel, Physikochem. Praktikum **11**, Berlin, 1926; Fresenius, Quant. Anal. I, 211 ff. (1903); G. Bruhns, Chem. Centr. **I**, 2768, etc. (1930).

apply to the method of Lundegårdh,[4] who determined small amounts of potassium and many other elements *spectroscopically* whereby he obtained a precision of about 5 per cent between 1 mg. and 0.001 mg. of initial substance.

6. If the determination form is a precipitate in the narrower sense of the word, its weight is often a multiple of the element (ion) to be determined. Naturally, under otherwise equal conditions, the precision is thereby increased, in fact, very considerably in a few cases. For example, the weight of ammonium phosphomolybdate is about seventy times as great as the weight of the phosphorus which it contains. On the other hand, a large tare (weighing tubes, filter tubes . . .) means a decrease in the weighing precision, as the weight of larger objects is more difficult to reproduce.

If extreme cases are disregarded, it may be stated that in quantitative microanalysis several milligrams of initial substance are normally used which correspond in general to several milligrams of determination form.

A decrease of the weight of sample either assumes an increase in the sensitivity of the balance or results in loss of accuracy.

Finally, it may be remarked that although most quantitative micromethods are reduced macro procedures, *it is not feasible* as a rule *to carry out a microanalysis only on the basis of experiences obtained in macroanalysis.* For one thing, micro work demands greater weight constancy of the objects to be weighed. In macro work it is sufficient if a crucible of 10-g. weight is weighed accurately to 0.2 mg.; microanalysis demands that a crucible one-half as heavy be constant, if possible, to within 0.002 mg. The ratio in the first case is therefore $1 : 2 \times 10^{-5}$ and in the second $1 : 4 \times 10^{-7}$. One must also not forget *that, particularly in quantitative work, the individual characteristics of the substances play a prominent part.*

7. One other important question is whether or not a reliable truly *representative sample* can be obtained in microanalysis. Benedetti-Pichler,[5] partly alone and partly with Professor B. Baule, worked on this problem, treating it experimentally and mathematically. As a principal result, it was stated *that the milligram method appears to be applicable with certainty with thorough mixing and with even a comparatively low degree of disintegration (particle size less than 0.02 mm.).*

[4] Lundegårdh, Die quantitative Spectralanalyse der Elemente, Jena, 1929. The sentence on p. 138 of this book, "... for most of the elements there are in fact no entirely reliable micromethods" is equivalent to a condemnation of quantitative microanalysis as a whole and is actually incorrect. See also Z. anal. Chem. **80,** 320 (1930). See Exercise 71.

[5] Z. anal. Chem. **61,** 305 (1922), **74,** 442 (1928). Further literature is cited in these papers.

III. Balances and Weighing in Particular

(a) The Microchemical Balance of Wilhelm H. F. Kuhlmann [1]

1. Construction and Use of the Balance

Various instruments meet the requirements, as stated, of permitting weighing with a precision of ± 0.002 mg. A large number of determinations have been carried out in my Institute especially by Dr. J. Donau (and also by the author) with the modified *Nernst balances*, and similar instruments have also rendered good service elsewhere. Anyone who has had practice in the use of the Nernst balance can, under certain conditions, save much time in weighing, and the author has therefore given necessary detailed instructions for this elsewhere.[2] At present every institute in which quantitative microanalytical work is carried out *must*, because of organic elementary analysis, have a microbalance of the type of Wilhelm H. F. Kuhlmann at its disposal, and since this instrument has a more general application because of its much wider weighing range, we will confine ourselves principally to its description.

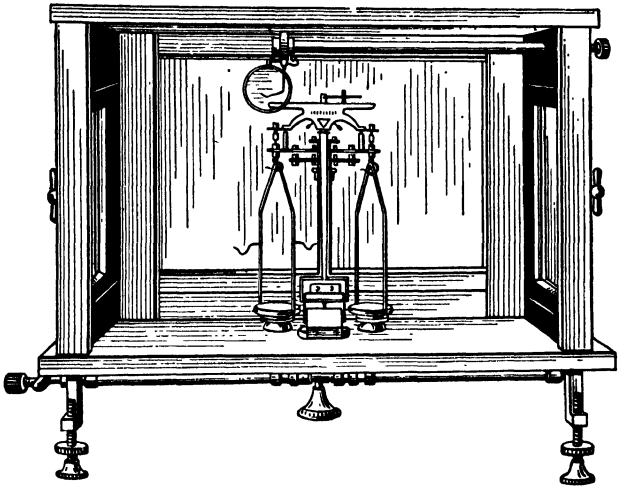


Fig. 42.—Kuhlmann microchemical balance. ($\frac{1}{5}$ natural size.)

The balance[3] (Fig. 42) consists of a solid brass beam of somewhat over 20-g. weight and 70-mm. length, the upper edge of which is

[1] Partly according to Pregl-Fyleman, Quantitative Organic Microanalysis, 2nd ed., Blakiston, Philadelphia, 1930, p. 6.

[2] Emich, Methoden der Mikrochemie, Abderhalden's Handbuch I, 3, p. 183. Berlin and Vienna, 1921.

[3] Obtainable from Wilhelm H. F. Kuhlmann, Hamburg, Steilshoperstr. 103. Recently other firms have put out balances of similar construction: Starke and Kammerer, Vienna IV; Sartorius, Göttingen; and others.

provided with notches for the rider. The beam swings on agate knife edges and planes. The sensitivity is practically constant to a maximum load of 20 g. The tip of the pointer as well as a small scale are observed by means of a magnifying concave mirror.

The rider edge has only 100 notches in all; since the rider weighs 5 mg., the balance must be adjusted so that it balances at zero when the rider is in the first notch at the left. Also its weight corresponds to 10 mg. when it is in the last notch to the right. In order to facilitate the setting of the rider, Pregl had a lens (unnecessary for short-sighted persons) attached to the rider carrier. In order to be certain that the rider falls into the lowest position in the notch, after placing it on the rider edge, it is repeatedly given a light push by the rider hook (and, of course, alternately from left and right) so that it swings for a moment each time. The edge is provided with figures 1 to 10 which signify whole milligrams. Milligrams and tenths of millgrams are therefore read from the position of the rider. Hundredths of milligrams are given directly by the deflection; thousandths are estimated. Kuhlmann adjusts the balance so that the deflection *difference* for $\frac{1}{10}$ mg. corresponds to 10 divisions of the pointer scale. Therefore if the pointer moves to 4.9 right and then 2.6 left, the pan at the left, which holds the material being weighed, is 2.3 hundredths milligrams heavier. If the rider is on the 5.8 mark the weight in milligrams is 5.823.

It is advisable in exact weighings to then move the rider over one notch to the right, in this case, therefore, to the notch 5.9. The deflection then should be such that a deflection difference of 7.7 hundredths milligrams in the opposite sense is obtained. If this is not obtained, the average of the two readings is taken.[4]

The following must be noted. If the arresting device is released (handle turned toward the back) the pans are first released. If these should begin to swing, as usually happens, the balance is arrested again and the procedure repeated until they practically do not move on lowering the arresting pins. Only then is the arrestment handle carefully turned until the stirrups and beam are free and the arrestment shaft cannot be turned any further. One soon learns how rapidly to release the beam so that it makes only very short swings. The first swings

[4] If a balance has been in use for a long time, it may easily happen that one division on the rider edge no longer corresponds to 10 divisions deflection difference. Two ways are open for overcoming this disagreement: either the ratio in question is determined by a series of readings of which the average is taken and a table is prepared for the deflection differences of 1 to, say, 87 which correspond to the values 1 to 100 thousandth milligrams, or the sensitivity is changed by turning the center of gravity nut; this latter operation, which requires some care, is carried out so that the screw is turned only very slightly each time, and care must be taken that the nuts which press against one another are tight.

after release are often not entirely regular and are therefore not noted. On the other hand, the fifth and sixth, or better, fifth, sixth and seventh swings are read off. It is best to consider the tenths of divisions (i.e., the thousandths milligrams) as units. If, therefore, the displacement as given above is 4.9 divisions to the left, one notes the number "49," by the next swing "26." Usually the next (seventh) swing is no longer "49" but perhaps "48" or "47." The average of the fifth and seventh readings is then taken, and the difference is then of course not "23" but "22." These calculations are so simple that one becomes quickly accustomed to carrying them out mentally while the balance is swinging. It need not be added that "deflection difference" is not the same as "zero point displacement"; the latter is obviously half of the former but is not considered any further here.

That deflections which are on the same side of the zero mark are to be added is understood.

It is not advisable to allow the swings of the balance to become too large.

In careful work the difference between two consecutive weighings will not be greater than 0.002 mg. ($=2\ \gamma$).[5]

2. Setting-up and Care of the Balance[6]

The balance should stand on a marble plate which rests on iron brackets built into the wall with a sheet of lead interposed between.

To protect the balance against accidental movements, the author has a hook fastened near the center of the base or on the bearing of the arrestment shaft (Fig. 43, p. 57). This hook is caught by a second hook on the upper end of a brass rod. The rod passes through a hole in the marble plate and is fitted at the lower end with a wing nut and lock washer. Fig. 43 shows a front cross-sectional view (in one-quarter natural size) of this simple device (which is also very useful with ordinary small analytical balances). B and B' are the bearings of the (not shown) arrestment shaft, GG' is the base plate of the balance, and CC' the marble plate.

Before setting up and also during use (e.g., once a semester) the balance must be thoroughly *cleaned*. This is especially necessary if

[5] One must not conclude from this that it is possible with this balance to actually weigh 20 g. exactly to 0.002 : 20,000 = 10^{-7}, since first of all the weights are at best exact only to 1 or 2 hundredths milligrams (change also slightly with time), and secondly the surface of many bodies is exposed to continual change and therefore hardly exactly reproducible. However, this does not change the correctness of the statement, which is fundamental for the application of the balance, that the accuracy of the necessary *difference weighings* is in accord with the requirements of the (centigram and) milligram method.

[6] See also E. Schwarz-Bergkampf, Z. anal. Chem. **69**, 321 (1926).

the arresting contacts stick, i.e., the balance does not begin to swing on release. The cleaning of these contacts, the knife edges and the bearings is most important. This is carried out by wiping with a small piece of chamois leather, which is held by forceps in such a manner that only the leather comes in contact with the agate surfaces. The cleaned parts are then examined with a good lens, watching especially for small hairs which may stick on a knife edge or on the tip of the pointer. Beam, rider edge and stirrups are brushed off, and the pans and bow strings wiped with a larger piece of chamois leather. The beam itself is touched as little as possible with the fingers; it is usually grasped by the upper part of the pointer, *particular care being taken with the tip of the latter.* Persons who are inclined to have moist fingers should clean their hands often during these manipulations. In place of the chamois leather (which is also not recommended by Felgentraeger) Dr. Benedetti-Pichler uses linen cloth which has been thoroughly and repeatedly washed and finally rinsed in distilled water. But then a *brushing off* must absolutely be the final cleaning, the brush having been cleaned of grease with acetone.

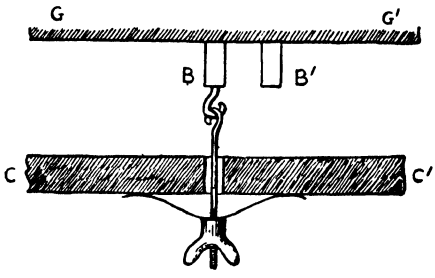


FIG. 43.—Fastening balance to table.

Before assembling the balance, the functioning of the beam and pan arrest is tested, any dirt which may be present is removed and if necessary the bearings of the shaft are lubricated with a trace of watchmaker's oil. The arrestment is then raised and the beam placed upon it. The beam naturally tips over to the right as the rider is lacking. By pressing lightly on the left side of the upper part of the pointer with a brush the beam may be tilted to the left. While it is in this position the left stirrup is placed in position. The pressure is carefully released and the beam assumes a horizontal position. Then the right stirrup is hung in place. Following this the pans are hung on the stirrups. These operations may be carried out very easily if the entire glass case *can be lifted off the base plate,* an arrangement which Kuhlmann supplies if desired. The balance is next leveled by observing the plumb bob and then fastened to the table (see above) and the functioning of the arrestment tested. It may also now be determined whether the pointer balances around zero when the rider is set on the zero notch. If this is not the case the wing nut is carefully adjusted. The removable case is convenient also for this.

The balance is allowed to stand for one hour with open doors to attain temperature equilibrium.

The case is then closed, the left hand holding the arrestment handle at the left, the right laid symmetrically at the right side so that the temperature of both sides of the balance case is affected in the same way. If the balance is now released, the zero point will generally differ from the one obtained before cleaning by a few hundredths milligrams. If the deviation is greater, the wing nut[7] must be adjusted and then one must wait again for some time. Usually further adjustment will not be necessary, and one can bring the zero point exactly to zero by turning the foot screws. The latter procedure is physically not entirely free from objection, but practically it is permissible and in any case most convenient.

A good normal eye can make a reading without any further assistance, and one soon learns to estimate the intervals (thousandth milligrams). For short-sighted (and not completely corrected) eyes the use of a "*telescopic magnifier*"[8] is to be highly recommended. There need be no fear then that the heat rays from the head of the observer will influence the readings to any appreciable extent.

Furthermore, the balance is tested as to the equality of the two arms of the beam. As a rule they are equal in length. The sensitivity with the maximum load of 20 g. is determined at the same time. If the balance arms are not equal, the ratio is noted so that it may be taken into consideration in those few cases in which it is necessary.

As first exercise the author would recommend the calibration of the 1-cg. weight of the set of weights. (The remaining weights are used only for tare purposes and therefore they need not, except for special reasons, be calibrated very accurately.) Since the *rider* is used oftenest, the centigram weight is referred to it as unity in the sense that its (the rider's) weight is assumed to be exactly 5 mg. (Exercise 65). Of course the values are set in a little table.

In weighing crucibles, absorption apparatus, etc., Pregl uses tare flasks (which can be obtained together with the balance) which he fills with *shot*. The procedure is as follows: the object is placed on the left pan and the empty tare flask and a 100-mg. weight on the right, and the balance released very slightly. Then medium-sized shot is added piece by piece to the flask until the beam tips over. The 100-mg. weight is then removed and further balancing carried out with the finest shot ("bird shot"). When the object finally is still about 1 mg. too heavy, the rider is used as already described. The author prefers taring with

[7] Some firms build the balances so that the adjustment of the wing nut is possible without opening the case.

[8] See, e.g., catalog "Med. 9" of Carl Zeiss, Jena and New York.

the set of weights or the tares mentioned below. In order not to confuse the pieces of equal weight, they are marked by the mechanician. (In the case of the decigram and centigram weights it is sufficient to bend over the end of the upright wire or the like.) It is advisable to prepare a second (and perhaps a third) centigram piece[9] so that the error of the set of weights is avoided in passing from, say, 4.59 to 4.60 g.

Tares, which are as much like the object to be weighed as possible in form and material, are *particularly suitable*, therefore, e.g., a porcelain crucible is used as tare for the porcelain working crucible, etc. If the tare is too heavy it can easily be brought to the proper, somewhat smaller, weight by grinding. If it is too light, the difference is made up by beads or rods of resistance glass. Tares and weights are kept in the balance case.

Before weighing, the balance case is again allowed to "acclimatize" for 10 minutes as described above, a reading of the zero point is then made and if necessary brought to exactly zero by manipulation of the foot screws while the beam is swinging.

The weighing is then carried out and after this, the zero point perhaps again determined.

All objects which are to be weighed must acquire the temperature of the balance case. If they have been ignited, they are first allowed to cool off in the laboratory, a crucible, e.g., on the copper block, etc. Then they are placed for 5 to 20 minutes, depending upon their size, in the neighborhood of the balance and finally for 5 to 10 minutes inside the case. The objects are never touched with the hand but always with a forceps, wire clamp or the like. Glass vessels (absorption apparatus, microbeakers) are first wiped off with a wet flannel and then with two dry pieces of chamois "until one has the feeling of gliding over smoothly" and then allowed to stand on a wire rack or the like (pen and pencil tray) for 15 minutes. In this way Pregl obtains a moisture film which is always reproducible.[10]

The following may be interposed at this point.

(*a*) Small, thin-walled porcelain crucibles[11] of 0.5- to 10-cc. capacity do not change noticeably in weight with repeated ignition in the Bunsen burner or in the electric muffle. The crucibles are allowed to cool as described above.

(*b*) *Platinum crucibles* of about 1 cc. capacity are cleaned by boiling in 1 : 1 nitric acid, washing and igniting until the flame is no longer

[9] Pregl recommends also among others aluminum tares of about 5-mg. weight. Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 16.

[10] For details on the handling of absorption apparatus see Pregl's book. The procedure of moist and dry wiping is due, as is known, principally to Wilhelm Ostwald.

[11] Obtainable from the Staatliche Porzellanmanufaktur, Wegelystrasse, Berlin.

colored yellow. They will thereby become a trifle lighter almost every time. After quick cooling the crucible is placed on a copper block by means of the platinum forceps, after 2 minutes placed on another block and finally allowed to stand in the balance case (p. 62) for 1 minute.

(*c*) For the temperature equalization of other vessels see pp. 70 and 73.

3. Further Remarks

1. In reference to the *balance room*, it may be said that its windows should face the north so that the balance is never exposed directly to sunlight. In the same way the *proximity* of ovens, flames and electric lamps is to be ayoided. Pregl recommends, as a source of artificial light, a ceiling lamp (600 candlepower). If the scale is not sufficiently illuminated, a little mirror is employed which can be held by a ball-joint on a stand. If the proximity of the wall has a disturbing effect, a thick sheet of aluminum is placed between the wall and the case as recommended by Felgentraeger. When the balance is not in use, the arresting handle is removed and reversed, so that the handle is *under* the base plate. Then the balance cannot be released by accidental touching of the handle.

If the balance is kept under a pasteboard protective case the latter must be removed several hours before the balance is to be used.

The interior of the balance is not dried but the introduction of a piece of pitchblende the size of a nut is useful in that electrical charges, which may create very remarkable disturbances in *very dry* air, will dissipate more rapidly.

2. In conclusion we mention briefly several experiences which E. Schwarz-Bergkampf (cited on p. 56) encountered in his comprehensive investigations. A weight constancy of $\pm 2\gamma$ can be attained only if the balance is placed in a room heated to constant temperature (as, e.g., by gas), in which the daily fluctuations of temperature are less than 1°. The temperature should be measured in the balance case. The weighings are constant only after the same temperature has prevailed in the balance room for several days; *this temperature should not be lower than 18 to 20° C.*, as otherwise the heating due to the body heat of the observer will quickly become noticeable. In tare weighings with a substance of equal density (a porcelain crucible is also used for glass) no further correction is necessary. With weights also no correction is usually necessary provided no long time interval (night) has elapsed. In the latter case, the difference in the buoyancy of the air must be considered. The change in the humidity of the atmosphere plays no essential rôle.

After long intervals between weighings (night) it is well to allow the balance to swing, arresting about five times. Between two weighings, but not when

weighing out the sample, the balance must be acclimatized at least 5 minutes because of the heating due to the observer. The thermometer behind the front window of the balance rises usually 0.1 to 0.2° C. during the weighing; if more than 0.3°, the equilibrium position has changed. The first swing is disregarded, the next two are correct. When arresting between these readings, only the beam is arrested, the pans remain free. If the weight changes continually during a weighing (mostly in the same direction) (heating by the material or the observer) one must leave the instrument and let the case stand open for 5 minutes.

The zero point is best determined *after* the weighing. If the balance room shows a constant temperature, the zero point is assumed to be constant, i.e., it is neglected.

The disturbances mentioned above are particularly noticeable in winter, as the rooms are heated only during working hours and the after effects of the temperature fall during the night seriously affect the equilibrium position of the balance. In the summer a favorably situated balance room is easily kept at almost constant temperature by careful ventilation.

The rider must sit perpendicularly, an inclination of merely 1° changes the weight about 6γ.[12] A magnetic field has no effect as the balance is built only of brass and German silver.

The use of a balance room which is kept at a constant temperature has the advantage of enabling one to work faster. Each weighing requires only half the time otherwise necessary, because the zero point determination is eliminated. The latter often gives a distorted picture, as double the possibility of error exists owing to the two weighings and the fact that the balance is somewhat warmed by the observer. Also the correct equilibrium position is reached more quickly and definitely at a constant temperature.

(b) Other Balances

It need not be mentioned particularly that, in cases in which a lower precision is required, the microchemist can use the ordinary analytical balance. It is especially used in preparative work. But other balances may also render good service especially if, in a series of experiments, the weighing must be as rapid as possible. We may mention for such cases the "*torsion spring balance*" of Hartmann and Braun, Frankfort-am-Main, the *Nernst balance* and the *projection spring balance*,[13] and finally for very coarse weighings (e.g., for preparative purposes) a simple glass thread (Salvioni) balance may be used which will be described briefly below.

[12] This calculation is based on measurements with a rider manufactured by Kuhlmann up to 1926. The amplitude of the error depends upon the weight of the rider, the distance between the resting point of the rider and its center of gravity and the angle of inclination.—TRANSLATOR.

[13] Emich, Methoden der Mikrochemie, Abderhaldens Handbuch I, 3, pp. 252, 255, Berlin and Vienna, 1921.

A glass sheet 9 by 12 cm. (Fig. 44) is set vertically into a baseboard. At the left is fixed a clamping device (shown in side view in detail drawing) which may be replaced by a cork sealed on the glass sheet. A ⌐-form glass rod is inserted into this in such a way that it may be turned with some friction. A glass fiber

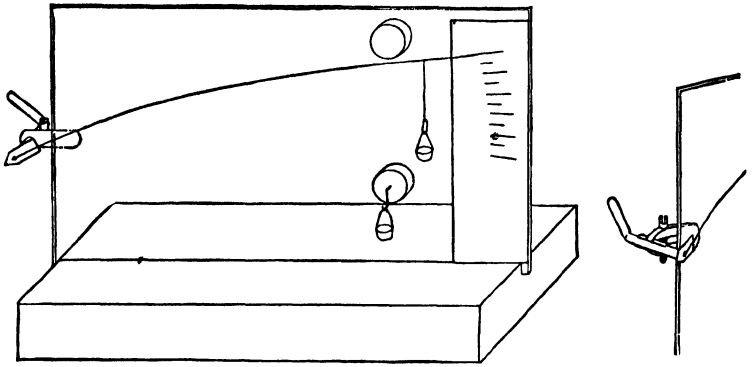


FIG. 44.—Simplified Salvioni balance.

0.1 mm. thick and 12 cm. long is fastened to it with sealing-wax. The free right-hand end carries by means of a quartz or tungsten fiber or Wollaston wire a hook on which either a platinum dish or an equally light platinum filter dish (p. 75) may be hung. The bottom of the latter is perforated and serves especially for filtering off crystals. The free end of the glass thread moves in front of a cardboard scale, which is empirically calibrated up to 50 mg. and permits estimation to half milligrams. When not in use the instrument is covered with a bell jar. The weighings are very rapid; setting up the apparatus requires little time and material, and it is not sensitive to vibrations or laboratory atmosphere.

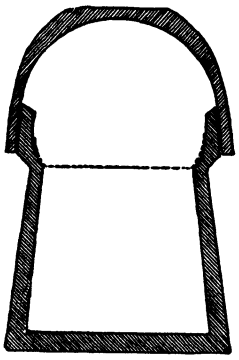


FIG. 45.—Micro hand desiccator.

IV. Drying

Desiccators and Drying Ovens

1. When working with very small vessels, a Canada balsam bottle (Fig. 45) is used as a *hand desiccator*. The drying medium is placed in the lower part, e.g., a stick of caustic alkali and several pieces of calcined lime (only the latter need be renewed often), the upper part contains a silver wire net (dotted line) which is *weakly* ignited now and then.

2. For larger vessels, the *ordinary desiccator* is used; in order that they cool off as quickly as possible a (possibly nickeled) copper block

(plate 1 cm. in height and 2 by 2 cm. at the base) is placed in the drying chamber. A similar but smaller block, about the size of a twenty-five cent piece, is placed in front of the scale of the Kuhlmann balance and a third beside the balance.

3. Pregl[1] uses the apparatus shown in Fig. 46 as a *tube* desiccator for drying substances in a combustion boat.

A tube 240 mm. long and 10 mm. in outer diameter, the bore of which is narrowed to a fine capillary for a distance of 20 to 30 mm. in the center, is filled on one (right) side with several layers of fine cotton followed by 50 mm. of granular calcium chloride. After this comes another layer of cotton, and finally the open end is closed with a tight-fitting stopper, through the hole of which a hair-fine capillary projects. This is widened at the other end and also filled with cotton to prevent the penetration of dust into the capillary. The other (left) half of the tube serves for the reception of the boat. The opening is closed by a calcium chloride tube which in turn is connected to a water pump. The corks *K* are filed flat on one side so that the apparatus cannot roll.

If the microdesiccator is to be *heated*, Pregl uses the "regenerating block"[2] (Fig. 47). The part of the tube containing the substance is placed in the wide channel of the block and rotation of the tube is prevented by pressing the two corks to the sides of the copper block. After the pump has been connected, the pressure in the desiccator falls to nearly the minimum which can be obtained in this way if the capillary is fine enough. The adjusting screw of the microburner permits regulation of the temperature. Before weighing, the pump is turned off, the desiccator removed from the heating apparatus, allowed to stand for a few

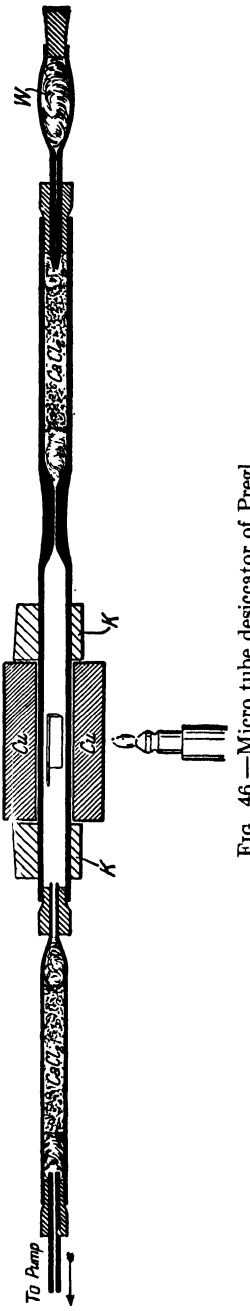


FIG. 46.—Micro tube desiccator of Pregl.
K, corks.
Cu, copper block.
W, cotton.

[1] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 67.

[2] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 69.

minutes until sufficient air has entered and then brought while still warm in the neighborhood of the balance. Here the calcium chloride tube is taken

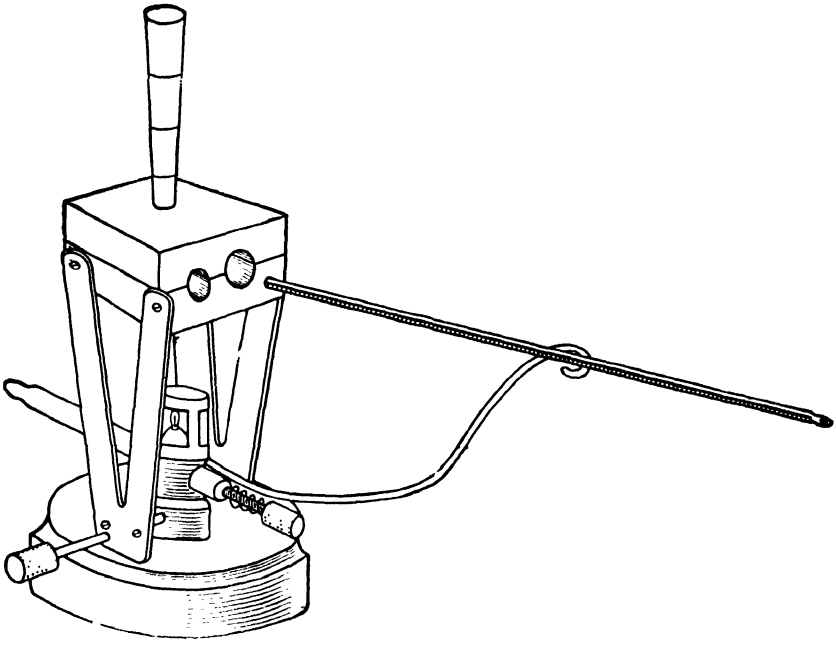


FIG. 47.—Pregl's regenerating block.

off, and the boat is drawn out with the platinum wire hook and quickly placed in the weighing bottle (Fig. 50) which has been held ready. After a few minutes' wait the weighing itself is carried out.

For drying the precipitate collected by a filter stick see p. 72.

4. In most cases the usual drying ovens will suffice, otherwise a simple drying apparatus can be improvised by placing a test tube in a flask of about 100-cc. capacity which can be heated on an asbestos-center wire gauze. A three-hole cork is placed in the test tube, in which are inserted a thermometer, one right-angle tube for introducing dried air, and a second for leading out the damp air. Crucibles, precipitating dishes and the like are either suspended from a hook fastened near the bulb of the thermometer or placed on a wire gauze.

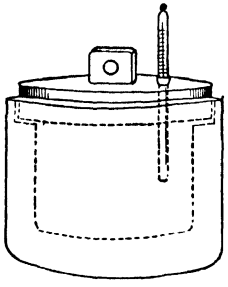


FIG. 48.—Small Stähler block.

(Block natural size, thermometer in half size.)

5. A Stähler *block*, of course of smaller dimensions, is also convenient. See Fig. 48, which needs explanation only in that the ther-

mometer shown is a "short thermometer." It is divided into divisions of 5° each, and therefore, although its range is 360°, it is only 8 cm. long. Such thermometers are sufficiently accurate for many purposes and are also very handy.

On drying at red heat, it may be said principally that platinum vessels are hardly ever heated directly by the flame but rather as a rule on a suitable base for which ordinary porcelain crucibles or their covers or especially quartz watch glasses of about 5-cm. diameter are particularly suitable.

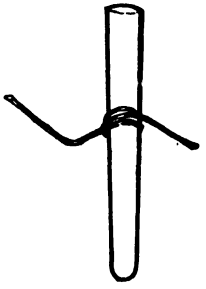


FIG. 49*a*.—Pregl weighing tube.

6. The weighing tubes (Fig. 49) described by Pregl [3] must be mentioned here.

The tubes can easily be made from drawn-out test tubes. The drawn-out tubes should have a length of 30 to 35 mm. and a diameter at the open end of 4 mm., at the sealed end, 2 to 3 mm. The stopper (Fig. 49*b*) is not ground in. For holding, either a thin glass handle or an aluminum wire of 0.5-mm. diameter wound around the tube will serve. The form of the wire may be seen from Fig. 49*b*; in reference to the tube *a* it may be remarked that the right end of the wire is bent downwards, the left first downwards and then

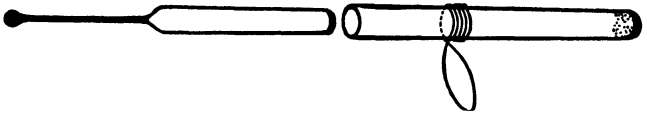


FIG. 49*b*.—Pregl weighing tube.

upwards. The tube touches the base at only three points, namely, at the sealed end, at the end of the short (right) arm of the wire and at the point where the long (left) arm is bent. If the tube must be touched with the fingers—perhaps for taking up a substance—it is not touched directly but with a piece of clean gauze. Of course it is then necessary to provide for proper temperature equalization.

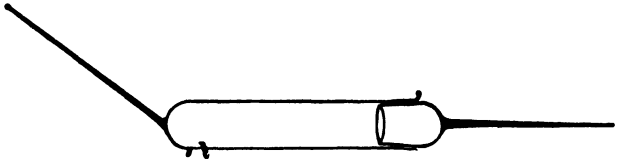


FIG. 50.—Weighing bottle of Pregl. Natural size.

7. If in a residue determination in a boat the substance used cannot be weighed open to the air, Pregl [4] uses a weighing bottle as shown in Fig. 50.

[3] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 100.

[4] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 67.

Pregl does not dry this bottle itself; it is kept in the balance case. If the substance has been dried in the boat it is placed as quickly as possible in the weighing bottle, which is then closed and allowed to stand for temperature equalization. See also section on "micro-muffle," p. 67.

V. Residue Determinations

1. By residue determinations we understand determinations in which a given substance is transformed into a second uniform substance without change of vessel and without wash or filtration operations. Such determinations are the simplest and therefore generally the most accurate analyses; also under favorable conditions they require only two weighings, beside the taring of the working vessels. One may also speak of a "method of three weighings."

The residue determinations are particularly simple in organic noble-metal salts such as chloroplatinates, also in chromates of organic bases, copper salts of organic acids, etc. With some metal compounds (copper valeriate and other copper compounds,[1] nickel dimethyl glyoxime and others) the volatility of the substance must be considered.

For the determinations one may use (*a*) porcelain crucibles of 0.5- to 5-cc. capacity; (*b*) platinum crucibles of about 1-cc. capacity; (*c*) dishes made of platinum according to the method of J. Donau (p. 75); (*d*) the platinum boats used in the micro elementary analysis. The vessels (*c*) and (*d*) permit the most rapid working by reason of their quick cooling, only one must make certain before using them (perhaps in a qualitative experiment) that no foaming occurs in the reaction involved. It may be mentioned in passing that this can often be prevented by the addition of asbestos (weighed with crucible).[2] The crucibles mentioned under (*b*) which weigh about 2 g., cool considerably more slowly, and the porcelain crucibles attain complete weight constancy only after about half an hour (see also p. 59).

In working with (*a*) and (*b*) we recommend the following precautions which are concerned primarily with the fuming off of organic salts of potassium, sodium, magnesium, calcium, barium and cobalt with concentrated sulfuric acid. The salt in question is moistened with two small drops of concentrated sulfuric acid, which are added from a dropping tube (capillary—see below). The crucible is covered and then heated from *above*[3] which Pregl[4] recommends be done

[1] See also especially H. Meyer, Analyse u. Konstitutionsermittlung, Berlin, 1922, p. 341.

[2] Benedetti-Pichler, Z. anal. Chem. **70**, 289 (1927).

[3] Fresenius, Quant. Analyse **I**, 81 (Braunschweig, 1903).

[4] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 163. E. Suschnig sometimes had difficulty in removing the last traces of pyrosulfate by means of ammonium carbonate. On the other hand, the addition

by playing the Bunsen flame on the cover of the crucible at intervals of 3 to 5 seconds. Of course one must be sure that the carbon is completely burned off; in the determination of sodium and potassium a piece of ammonium carbonate the size of a millet seed is dropped in the cooled crucible before the final ignition. The same end can be attained if the crucible containing the pyrosulfate residue is placed under a bell jar beside a dish containing concentrated ammonia.

In fuming off lead salts some nitric acid must be added to the sulfuric acid, in order to prevent the formation of lead sulfide, which might harm the platinum crucible.[5]

Pregl always ignites chromium salts in a porcelain crucible; on the other hand, the author has heated mercuric chromate innumerable times in Donau dishes [6] without noticing anything more than a pale tempering color at the place where the substance was in the crucible.

For heating ignition boats Pregl uses a glass tube of the following description:

2. *The Pregl micromuffle*[7] (Fig. 51) consists of a hard glass tube 200 mm. long and 10 mm. in outside diameter which is clamped at one end in a horizontal position so that it may be heated by the hottest part of a Bunsen flame, which is placed under it. The substance to be analyzed, which is weighed out in a platinum boat, is treated with a drop of dilute sulfuric acid (1 : 5) and pushed into the free end of the hard glass tube. In order to add the smallest possible drop of sulfuric acid and thereby prevent subsequent creeping, a capillary about 1 mm. wide, which has been drawn out hair-fine for several millimeters at one end and which must be held vertically when in use, is employed. Over the left end of the tube is fastened another glass tube of 15 to 17 mm. in outside diameter, which is bent at right angles, the longer arm being about 150 mm. and the shorter 50 mm. long. It is fastened by wrapping asbestos paper not too tightly inside the shorter arm and pushing it over the clamped end of the horizontal tube. A

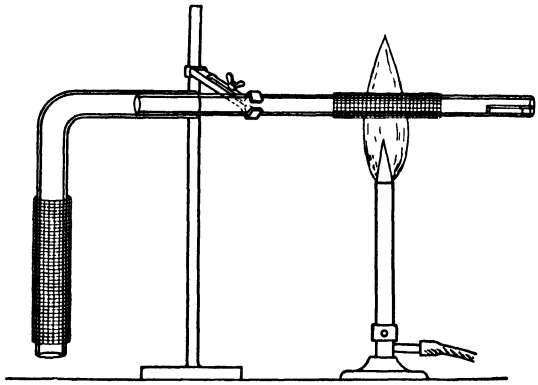


FIG. 51.—Pregl micromuffle.

of a drop of concentrated ammonia and a trace of alcohol gave good results. Monatsh. Chem. **42**, 401 (1921).

[5] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 164.

[6] Emich, Methoden der Mikrochemie, Abderhalden's Handbuch, Berlin and Vienna, 1921, Bd. I, 3, pp. 232, 268, 277.

[7] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 165.

wire gauze roll 100 mm. long is wrapped tightly around the long arm. If it is heated in a horizontal position and then turned to a vertical position, a continuous stream of air will rise and pass through the hard glass tube and over the boat. This stream of air can be continued at an unchanging rate by laying a Bunsen burner obliquely against the vertical arm of the tube so that the flame plays around the wire gauze roll. The hard glass tube is then heated at a distance of about 50 mm. from the boat by a silent blue Bunsen flame. Here also it is advisable to place an easily movable wire gauze roll about 50 mm. long to protect the tube. The distance of the flame and wire roll from the boat is important: if it is too great, the decomposition will take a long time; too short a distance will cause creeping of the contents of the boat and consequent loss of substance. After all the sulfuric acid has been driven off, which as a rule takes 5 minutes, at the most 10 minutes, the flame is rapidly moved toward the boat and finally the part of the tube containing the boat is ignited strongly for another 5 minutes. Thereby even in sodium determinations the subsequent addition of ammonium carbonate is unnecessary, as strong prolonged heating transforms the primary sulfate completely into the secondary sulfate with loss of sulfuric acid.

VI. Treatment of Precipitates

In the treatment of precipitates the various macro procedures have served as models. Recently we have preferred *those* methods in which the *precipitation vessel is weighed* together with the precipitate. The quantitative transfer of the precipitate is thereby avoided, and (at least in most cases) the "method of three weighings" (p. 66) is applicable. After various other experiments a simple and expedient method was found which consisted of weighing a little *immersion filter*,[1] which we call a "filterstick," with the precipitation vessel. This procedure works so quickly and accurately that it seems to the author that most other methods are dispensable.

(a) Working with the Filterstick

1. The apparatus is basically the same, whether the precipitate is dried at 100° or ignited, only in the first case the apparatus used is of glass and in the latter of porcelain, quartz or platinum. The glass apparatus can easily be made by the experimenter; for the rest the necessary hints are given below.

(*a*) *The precipitate is not ignited but dried at, say, 120°.*

For this case the apparatus consists of:

(α) The *microbeaker* (Fig. 52*a*) which is made from a test tube of Schott Geräte (Fiolax) glass; weight 2 to 3 g., capacity 5 to 10 cc.

(β) A hollow *glass stopper* with a thin handle (*b*), weight 1 to 1½ g.

[1] Stähler, Handbuch d. Arbeitsmethoden, Leipzig 1913, Bd. I, p. 680.

The stopper need not be ground in; it is, in fact, almost always dispensable in the case of non-hygroscopic materials.

(γ) A "stick" which is used for filtration. Weight ½ to 1 g. This stick can easily be made in the blast lamp of chemical resisting glass.[2] The handle is 70 to 90 mm. long and 2 mm. in outer diameter; the head is about 8 mm. long and about 6 mm. in greatest diameter. Jena glass combustion tubes are excellent. Short, thick-walled capillaries are drawn from a tube of about 6 mm. width. In this way a bulb-shaped

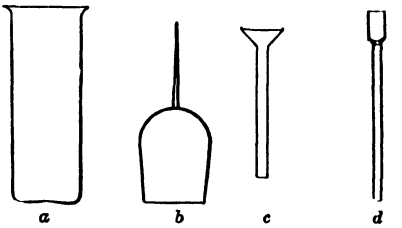


FIG. 52.—Microbeaker and filtersticks.

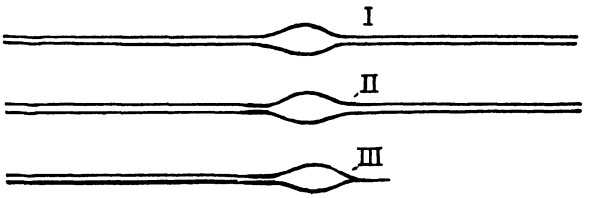


FIG. 53.—Preparation of filterstick.

widening forms between every two capillaries (Fig. 53), from which the head of the stick is fashioned by allowing the capillary to collapse at I by turning it in the pointed flame of the burner. Next the little pointed flame is directed to II, the glass well softened and then drawn out in such a way that the part II of the head becomes thin-walled. Finally the portion III is ground off, preferably on a rapidly turning Carborundum wheel. Finally the ground edges of the head and the end of the handle are fire-polished after thorough cleaning with water and alcohol.

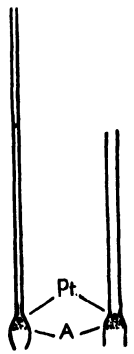


FIG. 54.—Asbestos-mat. Filtersticks (½-1 g.)

A little glass bulb or a platinum wire coil is placed at the point marked *Pt* in Fig. 54, to serve as a base for the asbestos layer, and finally the asbestos plug *A* is inserted, the density and thickness depending on the character of the precipitate.[3]

Filterstick and beaker may first be washed as recommended by Pregl, i.e., with hot chrom-sulfuric acid and

[2] Benedetti-Pichler, Z. anal. Chem. **64**, 409 (1924); Herm. Häusler, *ibid.* **64**, 362 (1924); E. Schwarz-Bergkampf, *ibid.* **69**, 321 (1926).

[3] Up to now the "chemically pure asbestos" of Hugershof, Leipzig, has been

water. That is, the acid mixture is placed in the beaker and the filterstick inserted so that the acid penetrates the asbestos and the whole heated for 5 minutes on the boiling water bath. After that the beaker is rinsed with hot water and the filter washed with suction as described below in the treatment of the precipitate. Then follows cleaning with hot nitric acid and water in the same way. Often the beaker is also steamed out for a time.

(*b*) *The precipitate must be ignited.* The apparatus is quite similar, only a thin-walled porcelain crucible of 5- to 10-cc. capacity is used in place of the microbeaker. The filterstick in this case is made of porcelain, quartz or platinum, or possibly of difficultly fusible glass. Beakers of quartz have not proved satisfactory up to now; it is difficult to bring them to constant weight (electrical charges?).

The quartz filterstick is made by fusing together two tubes in the gas-oxygen blowpipe. Its form is shown in Fig. 54*b*. It is filled with asbestos and prepared exactly like the glass sticks. The handle is 40 mm. long, the head 6 mm. The outer diameter of the handle is 3 to 4 mm.; of the head, 6 mm. Sticks made from Jena combustion tubes lose weight or are deformed after prolonged ignition at red heat.

Platinum sticks with Neubauer filter bottoms (Fig. 52*c*) may be obtained from Heraeus in Hanau-am-Main, Germany, or American Platinum Works, Newark, N. J. They weigh about 2.3 g.

Porcelain sticks are manufactured by the Staatliche Porzellanmanufaktur, Berlin. The filter bottom is made of unglazed porcelain.

2. *Use of the filterstick.* (*a*) *Weighing.* After cleaning, the stick is *dried* at a suitable temperature. For this the usual drying oven may be used. In order that the water drops remaining from the washing do not boil, the beaker and stick are first laid for a short time in the *open* oven. When the drops have disappeared, heating is continued for 10 minutes with closed doors, then the beaker and stick are allowed to cool, the stopper inserted if necessary and the beaker and stopper wiped off damp and dried as directed on p. 59. Then the little apparatus is allowed to stand for 10 minutes beside the balance and 5 minutes inside the case. Finally it is weighed accurately to 5 γ.

Another method of drying is described below.

If the sample to be weighed consists of individual loose particles (crystals), the filterstick may remain in the beaker. Otherwise it is

used with good results; with the small amounts required in the preparation of the filterstick other kinds may also be satisfactory. This is mentioned because Professor Pregl states that it is very difficult at present to obtain asbestos which shows constant weight in the filter tubes. For the procedure under discussion, this is hardly of importance since the necessary amount of asbestos in the filter tube is perhaps 10 to 20 times as great as that for the filterstick.

laid beside the latter on the balance pan, since pulverized or liquid material might adhere to the filter. Then it is again weighed accurately to 5 γ. The stick is laid on a clean watch glass while the material is dissolved.

(*b*) *Solution* and *precipitation* are carried out according to the directions tested in macroanalysis. The reagents must be tested for purity before use, and in case they show a turbidity they must be thoroughly centrifuged. For the dropwise addition of reagents a small long-handled pipet of about 2-cc. capacity, drawn out to a fine, fire-polished point, is used.

(*c*) *Filtration.* The asbestos layer in the filterstick is moistened with a drop of water (so that no thread falls off during further handling), and the stick then joined by a rubber tube to a suction apparatus *Pba* (Fig. 55) which is held in a retort clamp. If the washing is to be in the cold, the beaker is held in a second clamp held by the same stand as *P*. When washing hot, the beaker remains on the water bath. Since the glass is attacked by prolonged action of steam, the microbeaker is placed in a tall crucible or the like. In general, the filterstick is to be inserted in the beaker in such a way that it touches the bottom of the latter.

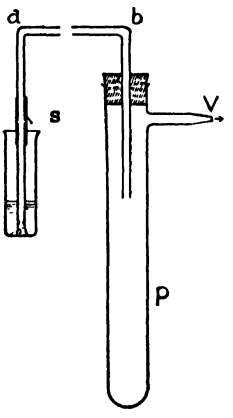


FIG. 55.—Filtration with the filterstick.

In many cases it is more convenient to hold the beaker *in the hand* during filtration; by carefully lifting the beaker, the liquid over the precipitate can be drawn off without the precipitate reaching the filter to any great extent; very fine precipitates would otherwise lengthen considerably the time of filtration by obstructing the filter layer.

To permit freedom of motion the piece *ab* is not made too short—10 to 12 cm. are usually sufficient. At *V* a rubber tube at least $\frac{1}{2}$ meter long is connected to which suction can be applied either with the mouth or water pump. The speed of filtration is regulated conveniently by inserting a T-tube in the connection *V*, the open end being closed with the finger as desired, whereby the desired suction is quickly obtained. For shutting off completely, pinch clamps may be put on the two tubes. When the precipitate has been freed from the solution, the wash liquid is sprayed into the beaker while the latter is turned in the hand and the walls rinsed off as thoroughly as possible. This procedure is repeated as often as required.

If the completeness of washing is to be tested, the receptacle *P* is

rinsed out, new wash liquid poured in the beaker, etc. It is still more convenient to place a special small vessel in the test tube P in which the filtrate is collected.

(*d*) *Drying.* (α) Nothing need be added to the discussion under 2*a* on drying in the usual drying oven.

(β) The following convenient and rapid procedure is due to A. Benedetti-Pichler.[4] The drying is carried out in a gas (air) stream in a very short time, and the apparatus is protected from dust. Fig. 56 shows the drying apparatus,[5] which has proved highly satisfactory in the most varied determinations. The drying tube T is held in the gas-heated aluminum block by the corks K. These are pressed against the block when the tube is laid in place. The friction on the sides of the block prevents rotation of the drying tube.

In order to insert the beaker and filterstick into the drying tube, the tube is taken out of the block and laid on the work bench. A flat surface on each of the corks K prevents the tube from rolling on the table.

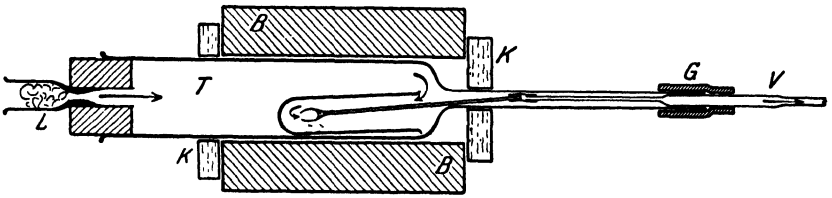


FIG. 56.—Drying apparatus of Benedetti-Pichler.

The rubber stopper at L is removed and the connecting tube V pushed so far into the drying tube that the end which is normally inside the drying tube projects from the mouth of the wider portion at L. This is connected by means of a small rubber tube to the end of the filterstick projecting from the mouth of the microbeaker. Then the connecting tube is grasped by the right hand and the filterstick is drawn by its help into the drying tube, while at the same time the beaker is pushed in by the finger of the left hand, so that the filterstick is always in contact with the bottom of the beaker. It is obvious that the part of the filterstick which touches the interior of the beaker must always be left in the beaker, otherwise some of the precipitate may be lost. Finally the stopper with the air filter L is inserted, and the vacuum pump, which is always connected with V by a rubber tube 2 mm. in

[4] Mikrochemie, Pregl-Festschrift, 6 (1929); see also Dick, Z. anal. Chem. **77**, 352 (1929).

[5] Obtainable from K. Schmitt, Graz, Lessingstrasse 25.

inner and 4 mm. in outer diameter, started. Only then is the drying tube placed in the block which is heated to the desired drying temperature.

The path of the air stream is indicated by the arrows. Naturally the rubber tube connection at *G* must be gas-tight. So that the connecting tube may remain easily movable, in spite of this, it is widened for only the short distance sufficient to make a tight connection at *G*. The connecting tube naturally must be longer than the drying tube. Furthermore, it is necessary that the piece of rubber tubing which joins the filterstick and the connecting tube must be outside of the heated part of the tube beyond the cork *K*. In order that the head of the filterstick may touch the bottom of the beaker at the same time, the handle must be at least 90 mm. long. A wad of long-fibered cotton is placed in the air filter. The bore of the air filter tube is narrowed to about 1 mm. A higher vacuum is not advantageous in the drying chamber because the wash liquid remaining in the beaker would vaporize too rapidly then and an undesirable condensate would form on all cool parts of the drying tube. If one wishes to increase the vacuum later, this may be done at any time by inserting a stopper with a fine capillary in the outer opening of the air filter. In a similar way, connection may be made with a suitable source of gas if the drying is to take place not in air but in another gas.

(γ) Work with precipitates which must be *ignited* is carried out in an analogous way. Weighing and precipitation take place, as stated, in a porcelain crucible, which need not be wiped off damp and dried; but, of course, care must be taken to provide for sufficient temperature equalization. (See also p. 59.) The platinum filterstick works rapidly, cleanly and accurately.

For ignition, the electric crucible (or muffle) oven of Heraeus is used. An unglazed porcelain crucible cover serves as base for the crucible. In place of the covers furnished with the crucible oven, which are lined with Chamotte, a large porcelain crucible cover is used to cover the ignition chamber. The chamber is cleaned occasionally with a fine brush. The temperature should never exceed 1100° in order not to produce an undesirable (and sometimes considerable) increase in weight of the crucible due to the spattering of the heating elements.

We usually take the crucible from the oven red hot and then stand it for 5 to 10 minutes on a clay triangle. It is then placed in a hand desiccator without drying medium in which the large crucibles must remain for 15 minutes; after this they may be brought in the neighborhood of the already *opened* balance case. After another 10 minutes the crucible is placed on the balance pan, the balance case closed, and after 5 minutes the final weighing carried out.

After wiping,[6] the beakers are allowed to stand for 10 minutes beside the balance and then 5 minutes on the balance pan in the closed balance, after which the final weighing may be made.

The beakers and crucibles used in the work should never stand around unprotected on the table but should be placed under a glass bell jar on a clean glass or porcelain base. We use wood blocks with a row of holes for the beakers, behind which is a second row of smaller holes which are inclined obliquely to the rear for holding the filter sticks (with heads upwards). These blocks are taken to the balance when weighing out, and the weighed beakers and filtersticks are placed immediately in corresponding holes in the block. In this way many samples may be weighed out at the same time without the danger of later interchanging the sticks, etc. (The holes for the beakers are best numbered.) If one works

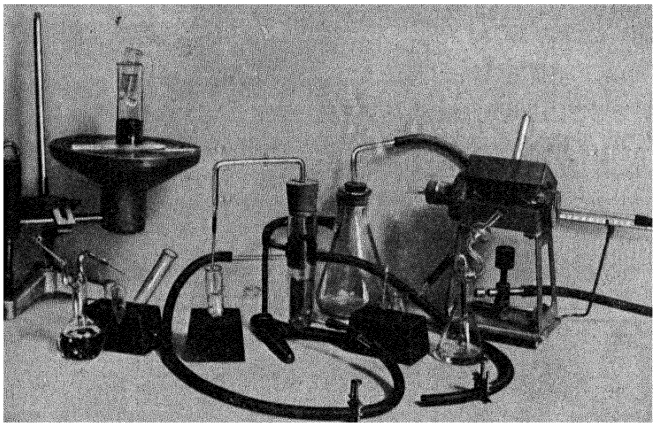


FIG. 57.—Apparatus for working with the filter stick.

Left Water bath with plate for heating the beaker. Pipet for addition of the reagent. Wash bottle. Centrifuge tubes for purifying the reagent. *Center.* Beaker and filterstick in filtration position Suction apparatus. Rubber-tubing connections. Wood block with beaker and filterstick. *Right.* Drying block of Benedetti-Pichler. Wash bottle

with a large number of crucibles, similar blocks are made for these. For holding filtersticks from the time of weighing until used, tall weighing bottles, narrow in form, are suitable. (In handling dried weighed filtersticks always keep the *heads upward.*)

A particular advantage of the filterstick method which may be emphasized is that it places smaller demands on skill and attention on the part of the worker than perhaps any other quantitative filtering method. As a slight disadvantage might be mentioned that one must naturally be particularly careful of *dust* which may be be present in the

[6] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 45; H. Häusler, Z. anal. Chem. **64**, 367 (1924).

laboratory; in the surroundings of this institute we have never found this nuisance to be a disturbing factor.

We include Fig. 57, p. 74, which was made from a photograph taken by Dr. H. J. Brenneis.

(b) Other Methods of Handling Precipitates

Numerous other methods included under this heading are copied from macromethods. We refer in particular to the procedure for the collection of silver chloride[7] and barium sulfate used by Pregl, for which one should consult his "Organic Microanalysis."

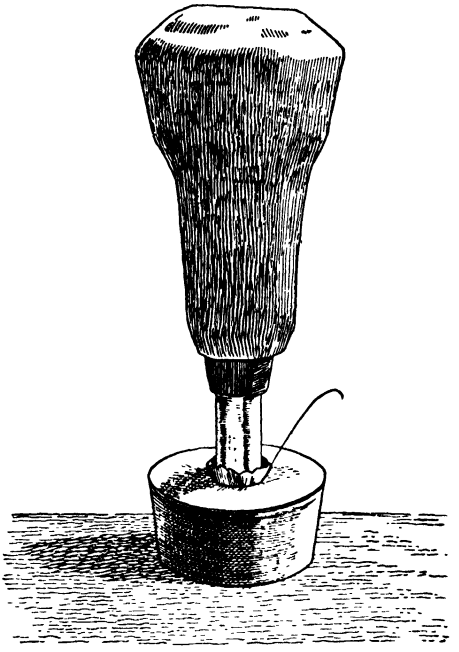


FIG. 58.—Preparation of Donau dishes.

Perhaps a few words may be said here on the Donau filter. These are little Gooch crucibles which one may make for himself out of platinum foil of about 0.004-mm. thickness by a stamping-out process which may be seen from Fig. 58. A glass rod can be used as stamping die and a rubber stopper for the base.

The bottom of this little dish is perforated with numerous fine holes; a filtering layer is made with asbestos or by filling with ammonium chloroplatinate and igniting. The Donau filters were originally designed for working exclusively with the Nernst microbalance. For this reason, their weight had to be kept small, and thereby several limitations are imposed on the methods.

Since the Donau method uses very small and light vessels, the weight of the precipitate always appears as the *difference between two relatively small numbers*, and therefore weighings on the Kuhlmann balance also may be carried out quickly and with very little attention to precautions.

Since the small vessels acquire constant weight very quickly, one must only take care that there is no change in the zero point of the balance, or at least give proper consideration to possible changes. An advantage not to be underrated is the low price of the material for the vessels.

[7] See also Stritar, Z. anal. Chem. **42**, 582 (1903); Hans Meyer, Analyse und Konstitutionsermittlung, Berlin, 1922, p. 52.

For these methods one requires the author's *filter capillary*,[8] Fig. 59, a glass tube ground flat on top, which is shown in the illustration with a paper microfilter laid on top (circular sheet with turned-up greased edge). It can be made without further description.

For suction, an *aspirator* is connected with a three-way stopcock and pump in such a way that either vacuum or aspirator suction may be applied at the point indicated by the arrow in Fig. 59. A third stopcock position permits filling the empty aspirator.

For further details the reader is referred to the literature; it may be remarked further that the platinum filter dishes (without asbestos filling) are especially convenient for the filtration of crystals in *preparative* micro work.

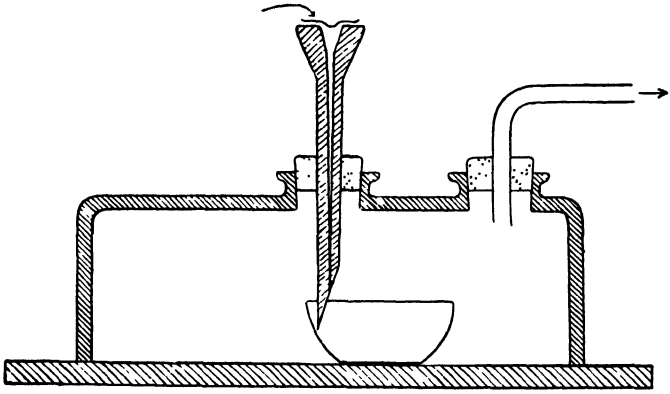


FIG. 59.—Filter capillary with suction bell jar.

Another method of filtering quantitative precipitates has been recently described by Kirk and Craig, Ind. and Eng. Chem., Anal. Ed. **3**, 345.

VII. Volumetric Analysis

One may differentiate between two types of volumetric micromethods, namely:

1. Those in which very dilute, e.g., 0.01 to 0.001 N, solutions are used in ordinary burets, and

2. Methods in which ordinary, e.g., 0.5 to 0.01 N, solutions are used in special (micro) burets which can easily be made from correspondingly narrow tubing. It is important among other things that they be cleaned very carefully before use.[1]

[8] Monatsh. Chem. **30**, 745 (1909). Obtainable from Vereinigte Fabriken f. L.B., Berlin N, Scharnhorststrasse 22. A similar but larger apparatus has recently been placed on the market by the firm of E. Leitz, Berlin. See also Emich, Lehrbuch d. Mikrochemie, Munich, 1926, pp. 89 f.

[1] For the theory, see F. L. Hahn, Z. anal. Chem. **80**, 321 (1930).

Recently various new forms of burets have been suggested, e.g., those with a stopcock on top by A. Benedetti-Pichler and by E. Schilow. *Weighing burets* also enjoy a growing popularity.

It appears to the author that for the purposes of the present book the method of Pregl will suffice, since it has the advantage that it requires scarcely any special apparatus. It is described in detail in his "Organic Microanalysis" to which the reader is referred,[2] since this work must be accessible to every microanalyst. For general orientation the following hints are given.

Ordinary pinch clamp burets of 10-cc. capacity divided into $\frac{1}{20}$ cc., for example, are used for *burets*. The outlet tubes are drawn out fine so that the end capillaries are 6 to 8 cm. long and have an outside diameter of 1 mm. For *titration solutions* Pregl uses either $\frac{1}{10}$ N or $\frac{1}{15}$ N solutions, depending upon whether they are for Kjeldahl or carboxyl determinations; on the other hand, 0.01 N solutions are preferred. In preparing the solutions Pregl starts with a 0.1 N hydrochloric acid and allows 50 cc. to flow into a 500-cc. volumetric flask and then fills the flask to the mark with water.[3] The alkali is standardized against the acid in essentially the usual way.

The indicator solution is prepared by dissolving *methyl red* (*o*-carboxy benzene-azo-dimethyl-aniline) in 0.1 N sodium hydroxide solution. The amount of alkali should not be sufficient to dissolve it completely.

The required amount of this solution is added by means of a glass thread to the solution to be titrated.

The titration is carried out in the usual way, the reaction being considered neutral when the (red) acid liquid has become a distinct canary yellow. The indicator permits working in artificial light. For numerous other methods see also the literature;[4] the potentiometric procedures deserve particular interest.

[2] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 114.

[3] This method naturally assumes that the 0.1 N solutions are standardized exactly so that the dilution to ten times the volume can be made without objection. On this see also the textbooks on quantitative analysis, e.g., Treadwell. The sodium hydroxide solution is prepared by Pregl according to Sörensen, i.e., highly concentrated, oily sodium hydroxide solution, free from carbonate, is diluted with water which has been thoroughly boiled and has been protected from contact with air containing carbon dioxide while cooling.

[4] In particular, perhaps: Benedetti-Pichler's reference work on the progress of microchemistry in the years 1915–1926; G. Klein and R. Strebinger, Progress of Microchemistry, Vienna, 1927, pp. 131–436; Bang, Mikromethoden zur Blutuntersuchung, Munich, 1922; Pincussen, Mikromethodik, Leipzig, 1930; Mandel and Steudel, Minimetr. Methoden, Berlin and Leipzig, 1924; the physiological chemist will refer primarily to Hoppe-Seyler-Thierfelder's Handbuch, Berlin, 1924. See among others also H. J. Fuchs, Mikrochemie **8**, 159 (1930).

II. PRACTICE EXERCISES

A. Qualitative Exercises

Exercise 1. Examination of a Powder, Separation of a Particle of the Mixture

One to two milligrams of minium are mixed in a mortar with one hundred times as much precipitated barium sulfate until the red particles are no longer visible to the naked eye. The mixture is kept in a bottle. The student receives about 1 mg. of this.

The sample is placed on a slide and there spread out in a thin layer with the clean preparation needle. The slide is placed under the binocular microscope, on the stage of which a piece of dull, dark paper has been placed if the instrument is not equipped with a proper background-plate. The left hand holds the slide between the index finger and thumb; as the preparation is moved, one can readily distinguish the little red particles. In order to pick them out, a *preparation needle* (p. 17) is taken in the right hand. The point of the needle is moistened with a trace of glycerine, for example, by rubbing a drop on the back of the left hand and drawing the needle over this spot. The point of the needle is brought under the objective (observation with naked eye), and then, while the observer looks into the microscope, the needle is carefully moved around until it appears in the field. It will appear indistinct and doubled over the preparation. It is lowered until it touches a red kernel, which sticks to it easily. Usually a few neighboring white particles are also taken up. The needle point is then lowered into a nearby drop of water and the particles drop off. This picking-out procedure is repeated five to ten times until a sufficient number of red particles have been removed from the mixture. Since they are still mixed with white particles a further separation is necessary. The water is first removed by holding a piece of filter paper or a glass capillary in the drop until it has been drawn off. Then the separation of white and red particles under the microscope continues, for example, by pushing one kind to the right and the other to left. For this purpose the dry (cleaned) preparation needle is used. Finally the white

powder is wiped away with a strip of filter paper and the following experiments may be carried out with the red particles:

1. Gentle heating on the narrow slide (p. 16) causes a temporary darkening in color; stronger heating, a yellow color, i.e., conversion into lead oxide.

2. Treatment with a droplet of dilute nitric acid causes a brown coloration. Examine each time against a white and a black background. The nitric acid solution is transferred to another part of the slide by means of a capillary tube, evaporated over the microburner, treated with copper acetate solution and used for the triple nitrite reaction described on p. 83.

Naturally other mixtures or objects can also be used in such experiments. The medical man will perhaps favor the stomach and intestines of a small animal which has been poisoned with arsenic. Mineralogy and geology present hundreds of examples. The experiment can be carried out with the ordinary, not binocular, microscope but requires more painstaking. The lowest magnification is chosen. If the objective can be taken apart (as for example Winkel [Göttingen] AB) then only half is used.

In many cases, immersion in a liquid which has an index of refraction approaching that of the principal constituent will render excellent service (private communication from N. Schoorl).

Recently, efforts have been made to apply microrgical methods to chemical work. Micromanipulators such as developed by Chambers,[1] Peterfi [2] and Taylor [3] are used to remove the microsample from the mass of other material and also to carry out identification reactions *in situ*. Although but little work of a purely chemical nature has been done in this direction, it has served to show that chemical microrgy has great possibilities.[4]

Exercise 2. Several Other Simple Experiments with the Microscope

(*a*) Calibration of the scale of the eyepiece micrometer, according to p. 7.

(*b*) Testing the behavior of the nicol prisms (p. 8). Determination of their orientation (p. 8, footnote 8).

(*c*) Determination of the behavior of a singly refracting crystal (sodium chloride) between crossed nicols, according to p. 9.

(*d*) Testing doubly refracting crystals; observation of the brightening caused, e.g., by a needle of urea (Exercise 3*b*).

(*e*) Observation of the extinction position:

(α) Parallel extinction. A kernel of oxalic acid is placed in a drop of 1 per cent manganous chloride solution on a slide, and stars of the form shown in Fig. 1 (p. 10) develop. When they are brought between

[1] Anat. Record, **24**, No. 1 (1922).
[2] Naturwissenschaften, **6**, 81 (February, 1923).
[3] Publications in Zoology, **26**, 443.
[4] R. Titus and H. Gray, Ind. and Eng. Chem., Anal. Ed. **2**, 368 (1930).

crossed nicols, the vertical and lateral arms brighten, the others remain dark.

(β) Oblique extinction. A drop of sodium chloroplatinate solution is allowed to evaporate on the slide and the crystals observed (Fig. 2, p. 10) as under (α). The angle of about 22° ("the extinction angle") can be read off with the help of the crosshairs (in the eyepiece) and the graduated scale (at the edge of the rotating stage). See also Appendix II.

(f) For pleochroism, see p. 10.

Exercise 3. Determination of the Index of Refraction by the Immersion Method

(α) Experiment with an *isotropic* body. Several milligrams of *sodium chloride* are placed on the slide according to p. 11 and covered with a cover glass: the particles have very sharp outlines. Some alcohol is allowed to flow under the cover glass by means of a capillary tube: the outlines are easily visible but weaker than before. Finally the alcohol is allowed to evaporate; it is replaced by ethylene bromide, whereupon the outlines almost vanish: ethylene bromide and sodium chloride have therefore approximately the same index of refraction. It is (for the sodium line at 18°) 1.54. An agreement to the second decimal is sufficient for the present purposes.

(b) Experiment with an *anisotropic* body. A fragment of a long crystal of urea about ¼ mm. thick and 1 mm. long is used for the experiment.

The two indices of refraction of urea (see also p. 11) are 1.61 and 1.485. In order to determine these the following experiments are carried out:

The object is placed in *oil of cloves* (or anisol), the index of refraction of which is 1.53 (1.56). The sample is covered as in the following cases with a (very small) fragment of a cover glass and brought under the polarization microscope (lowest magnification, condenser removed, stopped-down iris diaphragm, plane mirror). If the stage is rotated the "parallel extinction" is easily determined as in Exercise 2. The crystal is brought to the vanishing point between crossed nicols, and the upper nicol is removed. The microscope is carefully focused on a boundary line of the crystal and the drawn tube slowly raised. A bright "halo" of light can be seen at the boundaries of the crystal. This halo, known as the Becke line, moves into the medium of higher refractive index when the draw tube is raised and to the medium of lower index of refraction when the tube is lowered. In the present experiment the Becke line moves in a different direction depending on whether the polarized light passes the crystal as ordinary or extraordinary ray, i.e., *the refractive index for one ray (ordinary or extraordinary) is greater than 1.53 (1.56); for the other, smaller.* Then systematic tests with liquids having other indices of refraction follow. One will in this way obtain equality of the indices, i.e., observe fading of the outlines in question with castor oil (1.49) and with a

mixture of two parts of benzaldehyde and one part of carbon disulfide (1.61). Of course, the contours fade in castor oil if the principal edge lies perpendicular to the plane of vibration of the nicol, and in the mixture mentioned if it is parallel to it.

Exercise 4. Schlieren Experiments

(Compiled by Dr. H. Alber)

The following examples are carried out according to p. 40, and the observations are made with the schlieren microscope as well as with the unaided eye (visual method). In the following we use two capillaries with different internal diameters of the outlets; these are determined empirically for each system. The "narrow" capillary has a diameter of 0.14 mm.; the "wide" one, 0.17 mm.

(*a*) Static sample: distilled water; fluid sample: 0.2 per cent potassium chloride solution; wide capillaries; positive descending schlieren appear. On interchanging fluid and static samples, negative ascending schlieren are formed. The difference in refractive index (Δn_D) is 0.00025.

(*b*) Static sample: 0.3 per cent alcohol in water; fluid sample: distilled water; narrow capillary; the schlieren are negative and descending. If the fluid and static samples are interchanged, positive ascending schlieren are observed. $\Delta n_D = 0.00012$.

(*c*) Limit schlieren (schlieren which lie near the limit of possibility of observation) appear when, e.g., a 0.09 per cent potassium chloride solution and distilled water are alternately used as fluid and static samples; the wide capillary is used in this case. $\Delta n_D = 0.0001$.

(*d*) The fermentation experiments are carried out according to p. 43.

(*e*) A mixture, e.g., of benzene with 2 per cent *p*-xylol will serve for the crystallization experiments (p. 31); narrow capillary.

(*f*) The distillation experiments are carried out with the same mixture or with dilute alcohol (boiling capillaries!). The schlieren which appear when the distillate and residue flow into each other are observed, using the *narrow* capillary.

Exercise 5. Detection of Hydrogen and Hydroxyl Ion with Litmus Silk

In order to convert the litmus dye into a form suitable for microchemical analysis, the commercial dye is boiled with about double its weight of water, filtered, and treated boiling hot with excess sulfuric acid. Washed silk[1] is placed in the hot bath for about 30 minutes and then washed in running tap

[1] The raw silk is cleaned by boiling with soap solution and rinsing.

water, whereupon the color soon shades into violet. After drying, the preparation, the "red litmus silk," is stored in the dark.

In order to prepare the "blue litmus silk," a little water is poured over the red, a small amount of very dilute alkali is added carefully, then it is washed once quickly with distilled water, pressed between filter paper and dried. Since the blue silk obtained in this way loses its dye gradually when in water, it must be used only where very small droplets of liquid are employed. For less difficult cases, a blue silk is used which has been prepared by dipping the red in *lead acetate* and then washing.

The testing of the reaction of a solution is carried out as follows: a single, colored cocoon thread 2 cm. in length is fastened by means of a trace of Canada balsam or rubber cement to a small glass rod and cut off with a sharp scissors so that about 1 cm. remains free. This is drawn through a drop of alcohol to clean it, and the end is covered with a cover glass and examined under the microscope to make certain that it is perfect. A droplet of about 0.05 mg. of the liquid, the reaction of which is to be ascertained, is placed on a suitable base and the end of the thread is dipped vertically into the center of this droplet *so that it is exposed to the action of the solution while the latter is concentrating during the process of evaporation.* A preparation stand in which a ⊣-form glass rod with a thread at the lower end can be easily clamped is very convenient for this; however, a lump of wax fastened to the slide will also serve, as shown in Fig. 60.

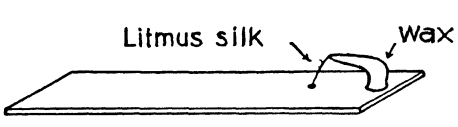


FIG. 60.—Experiment with litmus silk.

After the drop has evaporated, which takes place, as mentioned, at the point of the thread, the end to be examined is brought under the microscope and possibly cut off and made ready for another experiment. In order to avoid the action of the alkali content of the glass, the slide is covered with (neutral) paraffine or else quartz is used as a base.

The examination of the color is carried out with a magnification of about 100 times; of course, condenser illumination is used; daylight is preferred to an artificial source of light.

The exercise is carried out so that smaller and smaller amounts of acid and alkali are employed; in this way about the following limits of identification will be shown; for red litmus silk, 0.0003 γ sodium hydroxide; for blue litmus silk, 0.0005 γ hydrochloric acid; for lead oxide litmus silk, 0.001 γ hydrochloric acid.

Remarks: 1. A good exercise is the introduction of a little thread of red litmus silk into the spine of a nettle, e.g., *Urtica dioica*: the red litmus silk thread is fastened to the end of a glass thread as above and laid aside for a moment. Then

a spine is torn off with a forceps and placed on a slide under the binocular microscope; the lower wider part is cut off (with preparation needle or a small pocket knife) so that the (tubular) hair will be opened. The litmus silk is then brought into the field, the slide turned until the hair and thread point in the same direction and the latter pushed quickly in. For observing the color change, the preparation which hangs from the end of the glass thread is brought under the usual microscope with *condenser*. As a rule, the litmus silk will turn blue or violet in a short time.

2. When somewhat larger quantities of acid or alkali are involved, e.g., thousandths of a milligram (and less), a finely pointed narrow strip of good litmus paper or a sensitive tincture will serve the purpose.

3. Litmus silk is not recommended for projection experiments as the sources of artificial light are too poor in blue rays. If in a preparation the thread coloration by an acid is to be shown, a (white) human hair colored with some helianthine is used, the end of which is brought in contact with a trace of sulfuric acid.

Exercise 6. Neutralization

About 4 cu. mm. of dilute hydrochloric acid are placed in a centrifuge cone, to which some litmus tincture is added with a stirring hook. In a second centrifuge cone is some dilute alkali. Acid and alkali are about 0.1 *N*. The acid is neutralized by dipping the stirring hook in the alkali, mixing the droplet clinging to it with the acid by twirling, and continuing this until the indicator changes. If it is desired to neutralize hot, the apparatus shown in Fig. 5, p. 13, is used. In the neutral solution, one drop, as it hangs from the stirring hook after dipping in the alkali, must effect the change. The experiment may be projected using the cell, p. 15.

If the indicator cannot be added to the solution under consideration, the drop hanging to the stirring hook is tested by laying it like a rider on the edge of good litmus (azolithmin) paper.

Inorganic Cations

Exercise 7. Lead

I. Of the numerous microreactions for the lead ion the conversion to the *triple nitrite* $K_2PbCu(NO_2)_6$ is one of the most important.[1]

Since this is the first time we deal with a reaction of this type (crystal precipitation), the entire technic will be described in detail. It is important that the reaction be carried out with *varied* amounts of the

[1] Behrens-Kley, Mikrochem. Analyse, Leipzig and Hamburg, 1915 or 1922, p. 93. For the limits of identification of the individual reactions, see the tables in Emich, Lehrbuch der Mikrochemie, Munich, 1926, pp. 125–138.

sought-for ion in order to obtain a correct idea of how the reaction takes place and especially of the sensitivity.

We begin with, say, 0.2 mg. of a 1 per cent[2] (i.e., about 0.05 *N*) lead (acetate) solution taken from the stock bottle by means of the platinum loop (p. 22) and transferred to the slide by tapping. Next to it is placed ten times as much 1 per cent copper (acetate) solution and the two mixed with a glass thread which can be drawn for this purpose from a capillary. The mixture is evaporated (p. 16) on the slide, but it must not be boiled. The slide is then placed on a cold metal object (small anvil, copper block, base of the microscope, etc.) to cool off. The residue of the lead copper mixture is touched with a reagent prepared by mixing a drop of a mixture of equal parts of water, glacial acetic acid and 2 *N* ammonium acetate solution with a drop of a saturated aqueous solution of potassium nitrite.[3] A small drop of this reagent is brought by means of the glass thread on the evaporated lead copper mixture: the potassium-lead-copper nitrite separates immediately in the form of brown or black cubic crystals which may be as large as 10 to 25 μ but usually are smaller. They are observed with a magnification of 100 without the use of a cover glass. If one wishes to measure the crystals, the procedure described on p. 7 is followed.

The experiment is repeated with about $\frac{1}{10}$ of the given amounts using correspondingly dilute solutions; a definite incontestable reaction should still be obtained with only 0.01 γ lead. It is important that the drop to be evaporated does not spread over too large an area and that only a very small amount of reagent be used. If the amount of liquid to be evaporated on a small area is large, it can be drawn into a capillary tube, a small drop blown out on the slide, evaporated, another small drop placed on the residue and evaporated and so on.

In reference to the lead copper ratio, N. Schoorl [4] has found that the ratio 1 : 10 is the most favorable, and that 1000 : 1 and 1 : 300 were the *limit proportions* in which the reaction could be successfully carried out. In general, other ions do not interfere; on the other hand, the appearance of the brown crystals is hindered by the presence of 300 times as much mercuric chloride, and bismuth salts interfere in the same way, as the reader may easily ascertain for himself. With unknowns the reagent should *always be tested* before use.

II. *Precipitation of Lead as Sulfide, PbS. Other Lead Reactions.* 1. One to two milligrams of 1 per cent lead solution are placed in a centrifuge cone by taking a drop from the reagent bottle with the proper

[2] It need not be emphasized that the values given later in an analogous way hold approximately even if this is not particularly emphasized.

[3] See, e.g., Geilmann and Brünger, Glastechn. Berichte **7**, 328 (1929).

[4] N. Schoorl, Beiträge zur mikrochemischen Analysen, Wiesbaden, 1909, p. 23. Further literature, e.g., Behrens-Kley, Mikrochemische Analyse, Leipzig and Hamburg, 1915 or 1922, p. 67.

platinum loop (p. 22), touching it to the side of the cone and then centrifuging to the tip of the cone. First the acidity or alkalinity of the drop is determined by means of a pointed strip of litmus paper; if it is not decidedly acid, dilute nitric acid is added (stirring hook) and then hydrogen sulfide led in by means of the capillary (p. 23). The precipitation is complete in a few seconds;[5] the precipitate is washed, using the centrifuge, several times with hydrogen sulfide water and then once with pure water, which is finally removed as completely as possible. The precipitate is dissolved in about 1 mg. dilute nitric acid by warming (p. 13), and the solution is transferred to a slide by means of a capillary, finally evaporated and dissolved in 1 mg. of water. To hinder rapid evaporation the test solution obtained in this way can be covered with a small watch glass.

2. A sample of the solution is allowed to rise in a capillary tube which is then dipped into a potassium dichromate solution (drops on slide). In order to prevent too much of the solution from entering, the tube is drawn out into a point at the lower end. The upper end is closed with the index finger, which is raised sufficiently to permit the proper amount to enter. In order to complete the precipitation, the tube is sealed at both ends and centrifuged several times in reversed positions or the contents mixed with a glass thread. Finally the precipitate is washed according to p. 27, and possibly used for further reactions, e.g., the solubility in sodium hydroxide may be determined.

3. Another sample is diluted with water on the slide to a large drop, and some acetic acid and a minute kernel of potassium iodide are added. The drop is evaporated by heating until, on cooling, thin hexagonal plates of *lead iodide* are formed. They are recrystallized in the capillary according to p. 31. Hemmes prefers the lead iodide to the triple nitrite, the former being formed also from lead sulfate if this is moistened with a drop of water and a kernel of potassium iodide added (Böttger, private communication).

4. In a fourth sample the sulfate may be precipitated by dilute sulfuric acid, which is to be washed out in the same way. One part is used for the triple nitrite reaction. Here the solubility of the sulfate in ammonium acetate finds application.

5. *Exercise with the "Sulfide Thread."*[6] Guncotton [7] is repeatedly dipped

[5] It is recognized that the beginner often uses too much acid in the hydrogen sulfide precipitation and the precipitation is affected thereby; *this mistake occurs very easily in microanalysis*; therefore hydrogen sulfide water is added naturally after the introduction of the hydrogen sulfide.

[6] Liebigs Annalen 351, 426 (1907). See also Chamot and Mason, Handbook of Chemical Microscopy, New York, 1931, Vol. II, p. 376.

[7] A. Mayrhofer, Mikrochemie d. Arzneimittel u. Gifte, Berlin, 1923, I, 21, recommends that the statements made by E. Schmidt, Ausführliches Lehrbuch der Pharmazeutischen Chemie, 1910, p. 911, be considered.

alternately into about 15 per cent solutions of sodium sulfide and zinc sulfate, pressed out well each time and finally rinsed and dried. A thread should become deep black in a 1 per cent silver solution. If the end of the thread (see litmus silk, p. 81) is dipped into a *neutral* lead solution it should first become yellow. It becomes black in acid solution or on standing in the solution for some hours. The color also changes to black if the end is dipped into ammonium sulfide or in nitric acid diluted fifteen times (distinction from mercury with which the nitric acid causes no change at first in the yellow thread end). Hypobromite bleaches the thread; bathing in a drop of potassium dichromate solution produces then yellow lead chromate, which (distinction from bismuth) is not immediately colored black by alkaline stannous chloride. Limit of identification about 0.01 γ lead.

The precipitates which are on the end of the thread are *washed* by drawing the thread through drops on the slide. The drops must be taken suitably *small*, as otherwise disturbing solution action may take place.

We have mentioned the sulfide thread as a convenient reagent, but such thread reactions can also be carried out, e.g., by dipping the end of a cotton thread (after cleaning with acetone) into the (lead) solution in question, according to p. 81, and then exposing to the fumes of ammonium sulfide. (The guncotton has been recommended as carrier in its time because of its chemical resistance; however, as just mentioned other fibers may also be used.)

Exercise 8. Mercurous Mercury

1. Ten milligrams of the 1 per cent mercurous nitrate solution are treated with some dilute hydrochloric acid on the *narrow* slide. The drop is stirred with the glass needle and the whole cheesy precipitate separated from the solution by pushing it together with a strip of filter paper cut off evenly, which removes the liquid at the same time, and pushing the little heap to the end of the slide.

In order to obtain the "black precipitate" (mercuric amido chloride and metallic mercury), a tiny particle is touched with ammonia and, if necessary, observed with low magnification against a white and black background.

2. The rest of the precipitate is used in the *sublimation experiment*, according to p. 39. The sublimate is white and not distinctly crystalline with medium magnification. In order to convert it into the metal it is first boiled with a drop of soda solution on the slide. The black oxide formed sticks to the glass and can be washed by letting water from a wash bottle run over it. It is dried by gently heating, e.g., by holding high over a microburner, and again sublimed to a cold slide on which it appears as a fine gray efflorescence. It is brought under the microscope, scraped together (if necessary) with a glass thread and the *globules of mercury* recognized by their action as convex mirrors in reflected light.

3. The droplets of mercury are converted to the *iodide*: a kernel of iodine is pressed on a cover glass, and this is laid on the efflorescence. In a short time

appear the remarkable forms (worms, spheres, etc.) of the *red* (now and then also passing to the *yellow*) *iodide*. When carried out on somewhat larger scale, the test is particularly attractive under the binocular microscope.

4. The following conversion into metallic mercury is very simple, sensitive and characteristic. The mercurous (nitrate) solution, which may contain 0.5 γ mercury, is drawn into a capillary, and a piece of bare copper wire about 0.1 mm. thick and 1 to 3 mm. long is introduced. Then both ends are sealed, the wire and solution centrifuged to one end and heated for a few minutes in the boiling water bath (test tube). After opening, the wire is dried with filter paper and by means of forceps placed in a dry tube, of $\frac{1}{4}$-mm. bore, one end of which is sealed. In order to distil off the mercury, the glass at the end containing the wire is fused in the microburner until the wire is completely encased in the glass. The distilled drops are then visible under the magnifier. With very small amounts the search for the mercury drops is carried out, with the capillary filled with and immersed in cedar oil or water, against a dark background and with good reflected light. Confusion with air bubbles is not possible for experienced workers; one observes, for instance, that, with condenser illumination, the air bubbles have a bright center which becomes larger and smaller as the diaphragm is opened or closed, a phenomenon which of course does not take place in the case of mercury drops. The direct observation of the capillary with the binocular microscope is also recommended.

The test is successful with 0.2 γ mercury used in the form of mercurous nitrate, also when, say, 100 times as much silver nitrate is present.

Exercise 9. Silver

1. Of the numerous reactions of the silver ion, the formation and behavior of silver chloride are, as is known, particularly characteristic. About 2 mg. of a silver solution (1 per cent) are treated in a centrifuge cone with dilute hydrochloric acid. The solubility in ammonia and reprecipitation by nitric acid are tested. The precipitate is deposited by heating and centrifuging, washed twice with water, redissolved in ammonia and the solution allowed to stand on the slide. In order that the ammonia evaporate slowly, a small watch glass is laid over the drop. After about a quarter of an hour, a cover glass is placed on the preparation and it is examined with high magnification. Isometric crystals which show their strong refraction by their sharply prominent edges are formed.

The experiment is then to be repeated with a 0.1 per cent solution. A (micro) arc lamp (p. 92) is used for the detection of weak turbidities.

2. The formation of *silver dichromate* is mentioned because of the beauty of the reaction. Equal parts of 2 per cent silver nitrate solution and 10 per cent nitric acid are mixed and a kernel of potassium dichromate is placed in a drop of the mixture; splendid orange to blood-red crystals, rectangles, rhombohedrons and spears which may be as large as 2 mm. appear.[1] The sharp angles of the rhombohedrons vary: 43° (Behrens) 72° and 58–59° (Haushofer). The crystals are weakly pleochroic (Schoorl).

In order to convert *silver chloride* into the dichromate it is washed, fused to the slide and reduced by means of zinc and hydrochloric acid. The silver is washed first with acid and then with water, dissolved in nitric acid, the solution drawn away from the undissolved material if necessary, evaporated, redissolved in 5 per cent nitric acid, dichromate added and the drop allowed to evaporate if necessary.

3. The test obtained in 2 is used for a *permanent* preparation according to p. 48, section 3. Canada balsam in xylol [2] is used as sealing medium. If the preparation is to be projected in a lecture, it is instructive if the amount of silver used is stated, e.g., 10 γ.

4. The opportunity may be utilized to take a *photomicrograph*. The necessary instructions for this may be found in special books. See also Emich, "Lehrbuch d. Mikrochemie," Munich, 1926; or Chamot and Mason, "Handbook of Chemical Microscopy," Vol. I, New York, 1930.

5. For spot analysis see the literature.[3]

Exercise 10. Separation of a Mixture of AgCl, $PbCl_2$, and Hg_2Cl_2 (combined procedure by N. Schoorl)

(*a*) A mixture of about 10 mg. of 1 per cent solutions of lead, mercury and silver is precipitated on the slide with dilute hydrochloric acid, the precipitate washed several times with a little water and a small portion tested for mercurous ion with ammonia.

(*b*) The dried precipitate is heated on a corner of the slide over the microburner, according to p. 39, whereby the *mercurous chloride* is vaporized and (if 10 γ are present) forms a definite sublimate (even when it is mixed with a maximum of a thousand times as much of the two other chlorides). The remaining mixture should *not be heated to melting*. The testing of the sublimate is carried out as described above (Exercise 8, 2).

(*c*) The residue is extracted with boiling dilute hydrochloric acid; lead goes into solution and is identified by means of the triple nitrite reaction or as the iodide.

(*d*) The washed residue is extracted with ammonia and the latter allowed to evaporate. (See silver.)[1]

[1] Attractive projection experiment in Emich, Lehrbuch d. Mikrochemie, Munich, 1926.

[2] Obtainable in tin tubes.

[3] E.g., Z. anal. Chem. **74**, 380 (1928); Mikrochem. **7**, 411 (1929), **8**, 271 (1930).

[1] See also Benedetti-Pichler, Ind. and Eng. Chem., Anal. Ed. **2**, 309 (1930).

Exercise 11. Arsenic, Antimony, Tin

We will content ourselves here also with a small selection of reactions.

1. *Bettendorf Test.* One cubic millimeter of arsenious or arsenic acid containing about 0.1 γ arsenic is treated in a capillary with four times its volume of a solution of 1 part stannous chloride in 2 parts of fuming hydrochloric acid. The capillary is sealed at both ends, one end being drawn out into a medium fine, not too thin-walled point. The thoroughly mixed sample (p. 27), which should not fill more than half the tube, is heated as described on p. 14 in an amyl alcohol bath (boiling-point about 130°), i.e., 1 to 2 cc. of this liquid are placed in a test tube, the capillary dropped in and the alcohol heated to boiling. With the given amount of arsenic (relatively large), boiling should be continued for 1 minute; for smaller amounts up to 5 minutes. The capillary is then centrifuged with the point downwards and observed in the cell (p. 15) under low magnification. Limit of identification 0.02 γ As.

2. Ten γ arsenious acid are dissolved in several cubic millimeters of hydrochloric acid in a centrifuge cone by heating, diluted with water and hydrogen sulfide led in. The precipitate which has been washed by means of the centrifuge is transformed into arsenic acid by exposing to bromine vapors on a slide and the solution evaporated on a small area. (See lead iodide.) The arsenic acid is dissolved in excess ammonia and some ammonium nitrate and a kernel of magnesium acetate added. Dendritic, X-form and prismatic crystals of ammonium magnesium arsenate, $MgNH_4AsO_4 \cdot 6H_2O$, are formed, which cannot be distinguished *by its form* from the analogous phosphate (p. 102). But a differentiation is possible in that, if the precipitate which has been washed with ammonia is treated with silver nitrate, the arsenate becomes brown, the phosphate yellow.

If part of the precipitate adheres to the slide, as usually happens with dilute solutions, then it is only necessary to rinse it several times with water, dry it quickly and moisten with the silver solution. For observing the color it is advantageous with larger amounts of the precipitate to use a dark background and good reflected light (micro arc lamp). Single crystals and husks of the ammonium arsenate are observed with higher magnification in transmitted light.

3. For the detection of *antimony* a kernel of potassium iodide is placed in a drop, near the edge, of the 1 per cent hydrochloric acid solution of antimony (antimonous chloride or tartar emetic). On the opposite edge is placed a kernel of caesium chloride. As soon as the streams from the two salts meet, there begins the separation of orange-red six-sided plates, rosettes and stars, which are designated as "caesium iodo antimonite." Before this, one can often see the corresponding (colorless) chloro compound form. Bismuth gives a similar reaction, but the addition of alkaline stannite solution causes the formation of

metallic bismuth in the case of the bismuth compound, whereas the antimony compound remains unchanged or goes into solution. For a luminescence reaction of antimony see Exercise 16.

4. If a droplet of 1 per cent hydrochloric acid solution of *tin* (ic) is treated with a kernel of rubidium chloride, isometric crystals, predominantly octohedrons, of rubidium chloro stannate, Rb_2SnCl_6, are formed. But, as in many other cases, directly next to the reagent they appear as a fine dust.

Exercise 12. Examination of a Mixture of the Sulfides of Arsenic, Antimony and Tin

1. The precipitate is heated in a little test tube with 3 to 4 per cent hydrochloric acid until hydrogen sulfide is no longer evolved. It is then separated by centrifuging from the solution which contains essentially tin. The tin is identified by means of rubidium chloride. (See above.)

2. The residue is then treated with 25 per cent hydrochloric acid, dissolving the antimony, which may be identified by potassium iodide and caesium chloride.

3. Arsenic remains essentially in the *residue* (which may under certain conditions also contain traces of copper sulfide and mercuric sulfide). It is brought into solution by means of aqua regia or bromine (see above), the solution evaporated on the slide and the resulting arsenic acid identified as the ammonium magnesium compound.

4. Tin and antimony may also be identified in *one* sample by means of potassium iodide and caesium chloride: on careful examination the small colorless octohedra of the tin compound may be observed beside the red stars of the iodo antimonite.

For the methods which must be used when traces of one or the other ion are sought, see Emich, "Lehrbuch d. Mikrochemie," Munich, 1926.

Exercise 13. Analyses

Analyses are to be made of several alloys or mixed solutions of the metals discussed so far, e.g., type metal (Sb, Pb) or soft solder (Sn, Pb) the composition of which is unknown to the student.

The same procedure is followed in the other groups.

The student begins with the simplest case, in which the ions are mixed in approximately the same amounts, at first about 1 mg. of each; later he passes to several hundredths milligrams and also mixes the ions in unequal proportions.

Exercise 14. Ultramicroscopic Examination of a Colloidal Gold Solution

The study of colloidal solutions does not belong in the field of this book, but since the microchemist is often required to work with the ultramicroscope, the observation of a *colloidal gold solution* is recommended as an instructive and historically important experiment.

Ultramicroscopic particles can be made visible as is known by means of so-called *dark field illumination*, i.e., by illuminating the particles as strongly as possible by a source of light whose rays do not reach the object directly. Only the light "scattered" by the particles makes them visible (see also Fig. 61); the form and size of the particles are hidden thereby from the observer; what he can see are only the "diffraction disks."

There are various devices for dark field illumination. For the possessor of a large microscope the acquisition of a dark field condenser is recommended. Three types can be mentioned: (1) the paraboloid condenser, (2) the cardioid condenser, (3) the combination condenser. For most purposes the first one will suffice. As an object for study we recommend the *red gold sol* prepared according to Wolfgang Ostwald: 100 cc. (ordinary) distilled water plus 5 to 10 cc. of a 0.01 per cent gold chloride solution exactly neutralized or slightly alkaline with sodium or potassium carbonate are heated to boiling and a freshly prepared 10 per cent tannin solution added dropwise (1 drop every half minute) until an intensive red coloration appears. The solution may be kept for years after addition of some gum arabic and phenol.

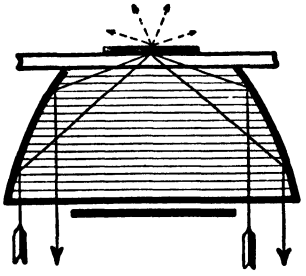


FIG. 61.—Path of rays in dark-field (paraboloid) condenser.

For ultramicroscopic observation a drop of the colloidal solution is placed on a very carefully cleaned slide and a clean cover glass placed over it. A drop of water is then placed on the *under side* of the slide and the latter laid on the condenser so that the drop of water forms an optical connection between the condenser and slide. Air bubbles in the object and this connection drop are to be avoided.

The slide and cover glass are previously cleaned by heating with chromsulfuric acid (cleaning solution), rinsing with water and twice with distilled alcohol, and allowed to dry. Do not wipe off.

A micro arc lamp or direct sunlight serves as a source of light; it is advisable to absorb the heat rays of the (parallel) beam by means of a 0.5 per cent copper sulfate solution in a cell placed before the illuminating plane mirror of the microscope. (See also Fig. 62.) The mirror is turned until a bright uniform spot is observed; raising or lowering the condenser helps to find this spot. If the microscope is focused on the liquid layer the diffraction disks can be seen in lively Brownian motion.

Also after careful cleaning of the slide and cover glass one can see a large

number of *quiescent* diffraction disks which arise from the impurities on the surface. A slide of the correct thickness, as given by the manufacturer of the condenser, should be used; it is important that the objective and condenser be designed for each other.

The higher magnification given on p. 5 suffices for the observations under discussion. The phenomenon is of course much more beautiful when a high-

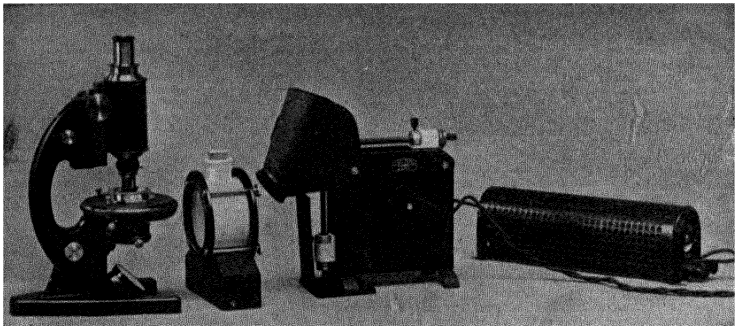


FIG. 62.—Set-up of ultramicroscope.

power eyepiece, e.g., the orthoscope eyepiece $f = 9$ mm. or the compensation eyepiece 18 of Zeiss, is used.

Exercise 15. Copper

1. We refer first to the triple nitrite reaction which has been discussed under lead. In order to utilize the blue coloration which cupric salts give with excess ammonia, 1 mg. of a copper nitrate solution containing 1 per cent copper ion is drawn into a capillary and some ammonia is allowed to enter. After the sample is mixed (p. 27), the solution, which appears colorless in thin layers, is centrifuged to the end and the tube cut off directly under the meniscus of the liquid. The capillary is then placed under the microscope as shown in Fig. 63 or 64. *W* is a little column of wax which holds the tube in a vertical position; *C* is a drop of water which forms an optical connection with the slide. Underneath this is the condenser; the microscope is focused on the upper end of the capillary. For the observation of the color it is best to have the meniscus of the liquid as flat as possible, a condition which

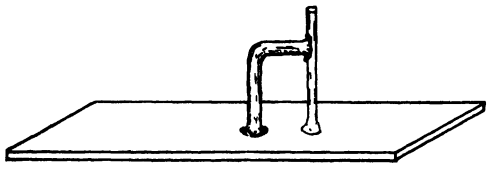


FIG. 63.—Axial illumination of colored solution.

can easily be obtained by addition of a drop of water (by means of the platinum loop, finely drawn-out capillary, etc.); a very small cover glass may be placed on top.

Those who wish to carry out such tests often may have a simple spring clamp made in which the capillary is clamped in a vertical position. Fig. 64 needs no further explanation.[1] The capillary may be coated with lampblack on the outside to keep out oblique light.[2] The smallest amount which may be detected depends upon the width of the capillary; one should not go below 0.2 to 0.5 mm., and in this case about 2 γ of copper are easly identified. The experiment is also suitable for projection.

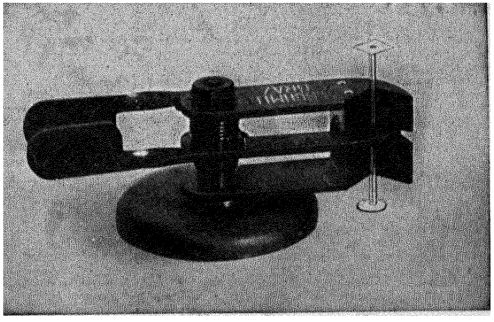


FIG. 64.—Capillary clamp for axial illumination.

2. Of the other copper reactions, only the precipitation with potassium ferrocyanide will be mentioned. It may be utilized in different ways; e.g., a cotton thread soaked in potassium ferrocyanide solution is dipped in the solution to be tested. An attractive projection experiment may be brought to the attention of the reader at this point. A crystal of potassium ferrocyanide the size of a lentil is thrown into a dilute copper solution in a small cell: "artificial cell." For the reactions of Spacu see the original.[3]

3. For spot reactions see the proper section.

Exercise 16. Bismuth [1]

1. *Detection by Means of Luminescence According to J. Donau.* Hydrogen (from a Kipp apparatus) which has passed through at least one wash bottle filled with moist porcelain shot is allowed to stream from a porcelain, quartz or platinum tube. The hydrogen is ignited; the flame should be no longer than half a centimeter. Then some *calcium carbonate* of the highest possible purity is stirred with some water to a thin paste and a small portion taken therefrom on a platinum loop, a platinum wire flattened somewhat at the end, a Wedekind magnesia

[1] Obtainable from K. Schmitt, Graz, Lessingstrasse 25.

[2] Ad. Mayrhofer, Qual. mikrochem. Methoden, etc., Abderhalden's Handbuch IV 7/C2, pp. 1145–1332, Berlin and Vienna, 1929, p. 1210.

[3] Z. anal. Chem. **64**, 331 (1924).

[1] This relatively unimportant ion is given extensive consideration, as it presents an opportunity to learn several interesting reactions.

rod or even a narrow strip of mica. The reaction itself is carried out in the following way.

The chalk mixture on the wire is ignited weakly in the flame, the solution containing the bismuth is added by means of a second loop and the chalk ignited weakly again. *If after cooling, the preparation is again held for a short time in the lower edge of the flame, a cerulean blue luminescence can best be seen at the moment it meets the flame.* As the chalk begins to glow, the luminescence naturally vanishes as it is masked by the yellow incandescence.

The sensitivity of the reaction is remarkable, as *the limit of identification is one ten millionth of a milligram* of bismuth. At this great dilution a dark room and blank test are necessary because the luminescence is no longer as intense. The blank test is carried out by placing a drop of water in a second chalk sample in a loop which has been previously weakly ignited, also held in the flame and compared with the first preparation. It is advisable to fuse both loops or platinum wires on which the reaction is carried out next to one another in one glass rod and to have the loops or wire ends as close as possible.

Manganese and antimony also give such luminescence reactions: with the first the luminescence is yellow, with the second greenish blue. Literature in Emich, "Lehrbuch d. Mikrochemie," Munich, 1926.

2. A kernel of potassium cobalti-cyanide produces a characteristic precipitate [1] in a solution of bismuth containing 12 per cent nitric acid.

3. For the detection of traces of bismuth by induced reduction of lead according to F. Feigl and P. Krumholz [2] see Exercise 78.

Exercise 17. Examination of a Solution in which Silver, Lead, Bismuth, Copper, Cadmium and Mercury May Be Present [1]

A trace of hydrochloric acid is added to the solution to be examined, which is slightly acid with nitric acid. If the solution remains clear, silver and mercurous ions are absent. If a precipitate (turbidity) appears, hydrochloric acid is added in small portions in order to obtain a complete precipitation of these ions, warmed [2] to hasten coagulation and centrifuged. The precipitate is examined further according to the usual methods.[3] The solution is precipitated with hydrogen sulfide, the precipitate extracted with ammonium or sodium sulfide, the resulting residue washed two to three times with 1 per cent ammonium nitrate solution and then sufficient 12 per cent nitric acid [4] added to dissolve completely. The sulfides are kept distributed in the dilute nitric acid by stirring with a glass thread and heated for 2 to 3 minutes on the water bath (it is best in the beginning to blow on a stream of air to facilitate the rapid removal of the evolved hydrogen sulfide). As a rule, it is possible to avoid in this way the sudden violent reaction of *small amounts of the sulfides* with the nitric acid with evo-

[1] A. Benedetti-Pichler, Z. anal. Chem. **70**, 257 f. (1927).
[2] Ber. dtsch. chem. Ges. **62**, 1138 (1929).
[1] A. Benedetti-Pichler, Z. anal. Chem. **70**, 257 f. (1927).
[2] Warming always on the water bath.
[3] E.g., N. Schoorl, Z. anal. Chem. **47**, 209 (1908).
[4] W. Böttger, Qualit. Analyse, Leipzig, 1925, pp. 426 and 429.

lution of nitrogen oxides and simultaneous formation of large lumps of molten sulfur which occludes the sulfides. The undissolved material is centrifuged off and treated further according to B.[5]

A. Solution

In the solution, lead, bismuth, copper and cadmium are to be tested for. The solution is evaporated if necessary to a smaller volume, treated with an excess of ammonia and warmed gently for a short time whereby any precipitate formed is better coagulated.[6] After centrifuging, the solution is analyzed according to the usual procedure for copper and cadmium. Any larger amounts of copper are evident immediately by the blue color, for the detection of cadmium one can, as in macro work, decolorize with excess potassium cyanide, remove dissolved cyanogen by warming in the air stream, centrifuge off any precipitate of *lead cyanide*[7] which may form and lead hydrogen sulfide into the clear solution.

The precipitate containing the entire bismuth in addition to the lead hydroxide is dissolved in a little 12 per cent nitric acid after washing with dilute ammonia, and the bismuth is separated as the basic nitrate [8] as follows: A watch glass of 4-cm. diameter is placed over the water bath and a strong fine stream of air directed to the center. The nitric acid solution is dropped on from a capillary and evaporated on the smallest possible area. The residue is moistened twice with 30 per cent nitric acid and evaporated each time to dryness in order to remove any chlorides which may be present. Then the residue is treated four times with a large drop of water and evaporated to dryness each time. The residue is extracted with a droplet of water and the lead may be tested for in the solution in the usual way. To test for bismuth, the basic nitrate is dissolved from the spot on the watch glass where the evaporated nitrates were, with the aid of a droplet of concentrated nitric acid, washed with a droplet of water and the bismuth detected in the nitric acid solution by means of a kernel of potassium cobalti-cyanide.[9]

[5] If any larger amounts of black sulfide (HgS) are left undissolved, it suffices for the detection of mercury, to dissolve in the least possible nitric acid (1 : 1), then to add an excess of concentrated ammonium oxalate solution. A bare copper wire is then dipped in and the solution warmed for several minutes on the water bath. The mercury separates out on the copper and may be distilled off in the capillary. See p. 87, and A. Stock and R. Heller, Z. ang. Chem. **39**, 466 (1926); see also Z. anal. Chem. **69**, 94 (1926); and N. Schoorl, Beitr. z. mikrochem. Analyse, Wiesbaden, 1909, reprint from Z. anal. Chem. **46, 47, 48.**

[6] W. Böttger, Qualit. Analyse, Leipzig, 1925, p. 234. The separation with ammonia according to N. Schoorl is excellent for the detection of small amounts of bismuth in the presence of much copper. In case no lead is present, some lead nitrate may be added in the detection of traces of bismuth in the presence of much copper before the precipitation with ammonia. The lead precipitation carries down the bismuth; it is also easier to work with the larger amount of precipitate.

[7] If no bismuth was present, small amounts of lead are in any case found only at this point. For the detection of lead in the precipitate, it is dissolved in concentrated nitric acid.

[8] Mikrochem. **4**, 40 (1926).

B. Residue

The residue from the nitric acid treatment consists principally of sulfur and possibly *mercuric sulfide*. Furthermore, it may contain small amounts of the sulfides of *lead, bismuth, copper and cadmium*, individual particles of which were protected from the action of the acid by a covering of sulfur. It may also contain *tin oxide* if the tin was originally present as stannous oxide and incompletely dissolved by ammonium sulfide, and finally the *sulfates of lead, barium and strontium*. For further treatment the material in question is divided into at least two parts.

1. The first part is examined for *mercury*. It is warmed with aqua regia and the solution carefully evaporated. (See above.) The residue is dissolved in water and either warmed at 60 to 80° for 10 minutes together with a small copper wire or allowed to stand with it over night. Stock recommends the addition of oxalic acid at this point. The amalgamated copper is rinsed off, dried and heated in a tube as described on p. 87. The sublimate may consist of droplets of mercury and also crystals of arsenious acid (from the reagents), which may make further separation necessary or may be identified by known methods. Mercury can be identified in this way if 0.01 mg. of it is present in the presence of a thousand times as much other metals. In many cases it is sufficient to treat the original solution directly with copper.

2. The other part of the residue is tested for the rest of the constituents mentioned above. Sulfur and mercury are removed by weak ignition in a porcelain crucible, and a new residue is thus obtained which may contain essentially the oxides of lead, bismuth, copper, cadmium and tin, and also the sulfates of lead, barium and strontium. For further examination it is to be remarked that the first five metals are not usually found here only, since at least some is always encountered where the metal is to be expected in the regular procedure. On the other hand, traces of barium or strontium may elude the analyst entirely if they are sought only by the usual procedure.

The residue is brought to dryness on the water bath with nitric acid and then extracted with dilute nitric acid.

(*a*) The solution contains lead, bismuth, copper, cadmium and is tested in the usual way.

(*b*) The residue may contain lead sulfate; it is extracted with ammonium acetate, copper acetate added and the triple nitrite reaction carried out.

(*c*) If the treatment with ammonium acetate also leaves a residue, it may first of all be tin oxide. It is brought into solution with concentrated hydrochloric acid and tested with rubidium chloride.

(*d*) The remaining undissolved material is assumed to be barium or strontium sulfate.

The test for tin oxide and for barium sulfate may also be carried out directly on the ignited residue from which we started out under 2.

[9] Some details which must be taken into consideration under certain circumstances could not be added here (and also not in the other separations); we recommend again that the reader consult Böttger's Qualitative Analysis or the other books cited in footnote, p. 3.

Exercise 18. Analyses

See p. 90.

Exercise 19. Cobalt, Nickel and Iron

1. A (moist) kernel of borax of about 0.2 mg. is touched with a platinum wire 0.1 mm. in thickness so that it sticks on the end of the wire. Then it is heated high over the microburner until the borax has swelled. The borax is touched with the 0.2-mg. loop, which holds a 1 : 1000 cobalt solution. The borax absorbs the solution. Then it is heated more strongly and finally at red heat for 1 minute. The bead is driven to the end of the wire in such a way that this is kept somewhat cooler than the part of the wire next to it. After cooling, the color can easily be seen with the magnifier (against a white background). Very weak colors in very small beads are observed by placing the bead in xylol or water and examining under low magnification and with condenser illumination.

2. Another pretty reaction consists of the formation of cobalt mercurithiocyanate, $CoHg(CNS)_4$. For this, Schoorl gives the following directions: 5 g. mercuric chloride and as much ammonium thiocyanate are dissolved in 6 g. of water by gentle warming, and the sample, which has been evaporated to dryness, is touched with this reagent (under the magnifier) without touching the slide (inoculation!) with the glass or platinum point. The least possible amount of the reagent is to be taken. Deep blue clusters of prismatic and often pointed crystals of the rhombic system are formed, together with irregular clumps and prickly spheres. (In the presence of zinc or cadmium, pale blue mixed crystals are obtained; copper also reacts with this reagent.) Small amounts of nickel do not interfere, but a quantity of this ion equal to the cobalt readily influences the beauty and form of the crystals. If ten times as much nickel is present they may not form at all. The cobalt compound easily forms supersaturated solutions.

3. *Nickel* is identified as the dimethyl glyoxime compound

$$(CH_3)_2 \cdot C_2(NO)_2Ni(CH_3)_2C_2(NOH)_2.$$

A kernel of dimethyl glyoxime is placed in an ammoniacal or neutral or weakly acetic acid droplet of the 1 per cent nickel solution on a slide and allowed to stand, perhaps covered with a watch glass, for a time. Rose-colored pleochroic needles are formed. The test works easily with 1 γ nickel. With crystals obtained in this way, the color change from red to yellow on rotating in polarized light is easily observed. Finally a sublimation experiment under reduced pressure with fractions of a milligram may also be carried out.

Cupric solutions give a similar reaction; these crystals are also pleochroic, and even the color change is similar; *but whereas the red color appears in the*

nickel compound when the plane of polarization of the nicol is perpendicular to the lengthwise direction of the needles, the behavior of the copper compound is just the opposite. Also, the crystals of the copper salt are not fine needle-shaped but more coarse, prismatic and of somewhat different color.

4. *Detection of Cobalt and Nickel in the Presence of One Another.* Since it is possible to detect one ion in the presence of an approximately equal amount of the other by the reactions given, it is advisable to carry out a separation when a large excess of one ion is present.

(*a*) In order to detect small amounts of nickel in the presence of much cobalt, the drop is treated with excess ammonia and shaken with air or allowed to stand loosely covered for several hours. The major portion of the cobalt is precipitated; the nickel is tested for in the solution with dimethyl glyoxime. One part nickel may be found in the presence of 5000 parts of cobalt.

(*b*) If, on the other hand, traces of cobalt are to be identified in the presence of much nickel, the former is precipitated in the form of the potassium nitrite double salt (very small cubes and octahedra), according to known directions, centrifuging, redissolving the precipitate in concentrated hydrochloric acid, evaporating and converting into the double thiocyanate or testing with borax.

(*c*) For further methods of identification see also the investigations of Kolthoff [1] and of Feigl; [2] also the section on spot analysis.

5. *Iron.* In order to determine the sensitivity of the Prussian blue reaction an iron chloride solution containing several millionths milligram ferric ion is concentrated on the slide according to p. 84 in such a way that the residue covers the smallest possible area. Then it is touched with a potassium ferrocyanide solution made acid with hydrochloric acid and brought under the microscope.

Exercise 20. Aluminum

1. *Alum Reaction.* (*a*) One milligram of the 1 per cent aluminum (nitrate) solution is evaporated on the slide, dissolved by breathing upon it and some powdered potassium hydrogen sulfate added. The octahedral alum crystals appear in a short time.[1] Rubidium and caesium alums are considerably less soluble and therefore appear in dilute solutions and form smaller crystals. In spite of this, Schoorl prefers the potassium compound.

(*b*) Ebler uses caesium hydrogen sulfate in order to combine the decomposition of silicates with the aluminum identification. One tenth milligram of kaolin is fused together with ten times as much caesium hydrogen sulfate in the platinum loop at dull red heat and the melt dissolved by boiling in a drop of water on a

[1] Mikrochem. **8**, 176 (1930).

[2] Österr. Chem. Ztg. **26**, 86 (1923).

[1] Attractive projection experiment: about 0.1 cc. of a solution of crystallized aluminum nitrate 1 : 3 and powdered $KHSO_4$ is used. In case (supersaturation) the crystallization does not begin within a reasonable time, the preparation is seeded with a trace of caesium alum which is introduced with a platinum needle.

narrow slide. The solution is then drawn off from the undissolved matter and allowed to crystallize.

2. *Fluorescence Reaction According to Goppelsroeder.* The reagent is prepared by either dissolving *morin* or boiling *fustic wood shavings* in 20 per cent alcohol in about the proportion of 1 part of fustic wood or morin to 10 or 10,000 parts, respectively, of solvent.

(*a*) If 1 cc. of a 1 : 1000 aluminum solution is treated with several drops of the reagent, a strongly green fluorescent solution is formed. The observation is carried out best by so concentrating the light from an arc lamp by the aid of a collecting lens that the image of the positive crater appears in the interior of the liquid. Limit of identification 0.025 γ aluminum.

(*b*) For small amounts of liquid a capillary is used which can be illuminated

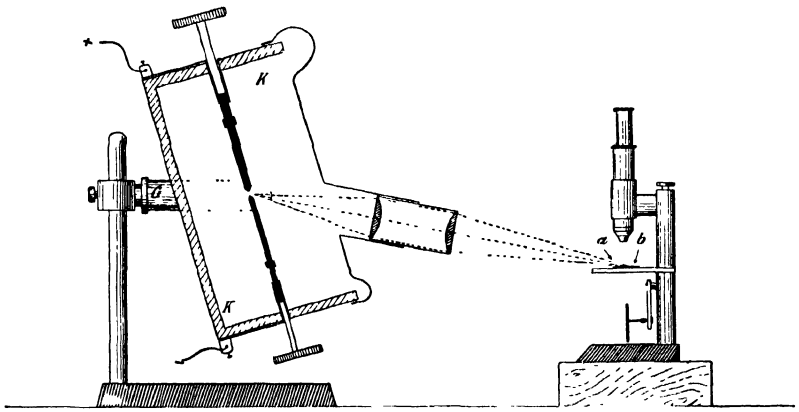


FIG. 65.—Testing for fluorescence and fine turbidity.

as shown in Fig. 65. (In place of the lamp illustrated here, the micro arc lamp shown in Fig. 62, p. 92, can be used, and considering the low use of current, is recommended.)

K is an asbestos-lined wooden box which is mounted in a horizontal fork *G*. On one wall is placed an ordinary collecting lens, adjustable in a tube which delivers parallel or converging light, depending upon its distance from the arc light.

The capillary *ab* is placed on the stage in such a way that no disturbing reflected light enters. One can obtain a decided fluorescence with this arrangement with several millionths of a milligram, but one must not omit testing the reagent (diluted with the corresponding amount of water) for a possible fluorescence of its own.

In general, the other cations do *not* disturb the fluorescence reaction, espe-

cially not the rare earths, as Goppelsroeder had already discovered. On the other hand, high concentration of hydrogen ions interferes, that is, free mineral acids must be buffered with sodium acetate before addition of the morin solution.

3. The alum and fluorescence reactions may be combined by separating the crystallized (caesium) alum from the mother liquor by means of filter paper, then dissolving in water, adding fustic wood solution, etc.

4. The attention of the reader is called to the reaction of Atack: Emich, "Lehrbuch d. Mikrochemie," Munich, 1926, p. 165. Original in J. Soc. Chem. Ind. **34**, 936 (1915). See also Hammett and Sottery, J.A.C.S. **47**, 143 (1925).

Exercise 21. Chromium

1. One milligram of a chromic solution containing 1 γ Cr is evaporated on the narrow slide and fused with five times as much sodium peroxide. The yellow color can be observed immediately. For confirmation of chromium, the melt is evaporated twice to dryness with water, acidified with dilute nitric acid and a kernel of silver nitrate added. Properties of the crystallization are described on p. 88.

2. The detection of chromic acid by means of diphenyl carbazide is very sensitive.[1]

For example, the melt obtained in 1 is dissolved in dilute sulfuric acid, the solution placed on a suitable base (white porcelain plate) and an alcoholic solution of diphenyl carbazide added. The appearance of a red-violet color shows the presence of chromic acid. See spot analysis.

Exercise 22. Zinc

1. A drop of a 1 per cent solution of zinc is precipitated with hydrogen sulfide after possible addition of sodium acetate and the zinc sulfide washed with hydrogen sulfide water. It is then dissolved in dilute nitric acid, the hydrogen sulfide is driven off, and the solution (A) is used in the following experiment.

2. *Crystalline Precipitation of Zinc Ferricyanide.* This very characteristic and sensitive reaction requires a rather dilute solution if definite crystals are to be obtained. A kernel of potassium ferricyanide is placed in solution A, which is strongly acid with nitric acid. If a yellow turbidity appears around the kernel the presence of zinc is possible. If the crystals cannot be recognized as such at a magnification of 400 times, solution A is diluted five times with water and the test repeated until the yellow *quadratic crystals* which show the presence of zinc can be seen. A 0.1 per cent solution of zinc containing 7 per cent nitric acid gives beautiful crystals.

3. For other zinc reactions, e.g., sodium zinc carbonate (colorless tetrahedra), see also the "Lehrbuch."[1] As a rule, zinc may be driven easily out of alloys,

[1] F. Feigl and P. Krumholz, Mikrochemie, Pregl-Festschrift, p. 80 (1929).

[1] Emich, Lehrbuch d. Mikrochemie, Munich, 1926, p. 167; Benedetti-Pichler, Z. anal. Chem. **70**, 257 (1927).

e.g., brass by heating in a difficultly fusible capillary. The sublimate consists, if little alloy is used, essentially of the oxide. It is also possible to sublime in a vacuum.

Exercise 23. Manganese

1. The manganate fusion is carried out on the narrow slide or in the platinum loop with sodium peroxide as the reagent. In the second case, one must be careful that not too high a temperature is reached. In the first case, the possible manganese content of the slide must be taken into consideration: the necessary blank and practice experiments are obvious from these hints.

2. If one wishes to utilize microchemically the oxidation of the manganous ion to permanganic acid by means of lead peroxide and nitric acid, it is possible to test very dilute solutions which have been clarified by centrifuging according to p. 92. A few hundredths γ manganese can easily be identified. The oxidation by means of ammonium persulfate and silver nitrate has also proved satisfactory in observations up to now.

3. For the luminescence reaction of Donau, see p. 93, and Emich, "Lehrbuch d. Mikrochemie," Munich, 1926, p. 153.

4. The permanganates form *mixed crystals* with perchlorates. One milligram of perchloric acid (commercial reagent of 1.2 sp. gr., or the corresponding amount of sodium perchlorate) is placed in a large drop on the slide, and then in two places not too close to one another a kernel each of potassium permanganate and rubidium chloride are added to the drop. After 5 to 10 minutes a copious crystallization of the mixed crystals, $RbCl(Mn)O_4$, which are of widely different color intensity, appears.

For *microspectroscopic* experiments, see also Emich, "Lehrbuch d. Mikrochemie," Munich, 1926, p. 62. For spot reactions, see the proper section.

For the detection of the ions of the ammonium sulfide group in the presence of one another, a procedure such as used in macroanalysis will serve as a rule if centrifuge cones are used principally.

Exercise 24. Calcium

1. *Identification as Gypsum:* $CaSO_4 \cdot 2H_2O$. Some sulfuric acid is added to 2 mg. of a 1 : 200 calcium solution on the slide, evaporated and finally heated until no more sulfuric acid fumes are evolved. (A quartz dish is better than a slide, as the glass easily gives up some calcium.) The residue is dissolved in water to which some acetic acid is added and allowed to evaporate. The gypsum crystals obtained are, to be sure, often somewhat varied but still very characteristic. With rapid evaporation (which should be avoided), chiefly bundles of needles are formed.

On the other hand, with slow evaporation are formed: (*a*) leaflets with rhomboidal outlines and sharp angles of 53° (often), 66° or 28°, (*b*) twins, recognizable by the interior angles of (mostly) 104, 103 or 76°.

The crosshair eyepiece and the calibrated (rotating) stage are used in the measurement of the angles. First one and then the other arm of the angle is made to coincide with one of the crosshairs, the angle read off each time from the edge of the stage and the difference between the two readings taken; naturally the direction of rotation must be noted. The measurements are repeated on other crystals and the average of the observation taken. See Appendix II.

2. Other forms of identification: Gay-Lussite, tartrate, oxalate, potassium ferrocyanide, are described elsewhere, as also barium and strontium reactions and the separation involved.[1] Rosenthaler prefers (private communication) hydriodic acid, which reacts also with the oxalates and sulfates.

Exercise 25. Magnesium

1. *Ammonium Magnesium Phosphate.* One milligram of an acidified 1 : 1000 magnesium solution is exposed to the fumes of ammonia by inverting the slide over a reagent bottle containing some 20 per cent ammonia. (Since the reagent will also absorb acid from the drop, this bottle should not be used for other purposes.) Then a kernel of microcosmic salt is placed on the edge of the drop and the slide again placed over the reagent bottle. In the immediate neighborhood of the phosphate, dendritic crystals are formed; farther away there are always better-formed crystals, especially six-armed stars, X-forms and prismatic crystals. The crystals belong to the rhombic system, are weakly polarizing and do not differ much in refractivity from the solution (therefore observe with small iris diaphragm opening!).

Potassium and sodium do not interfere even if they are present in an amount 250 times that of the magnesium. (In the presence of lithium, which is not considered here, the magnesium must be separated by means of barium hydroxide.) Finally, calcium may be kept from interfering by the addition of citric acid, if it does not exceed the amount of the magnesium 50 times (Schoorl).

Various limits are given for this reaction. With 0.05 γ it becomes usually uncertain. In limiting cases it is important that no ammonia salts be added, starting therefore from an approximately neutral solution. The residue can be redissolved after evaporation by breathing upon it and then examined again under the microscope; it is then possible (assuming proper practice and care) to detect very small amounts.

2. For detection and colorimetric determination of magnesium according to F. L. Hahn, see Exercises 74 and 82.

[1] Emich, Lehrbuch d. Mikrochemie, Munich, 1926, pp. 171 f.

3. In reference to the *detection of calcium and magnesium in the presence of one another*, it is possible when approximately equal amounts are present (as in dolomite) to easily identify calcium as gypsum and magnesium as ammonium magnesium phosphate if the calcium is identified in one drop as sulfate and a second drop tested for magnesium in the presence of citric acid. Naturally the usual procedure of separation with ammonium carbonate may also be employed. It is to be noted that the presence of much ammonium salts renders the precipitation of the calcium group incomplete; these, therefore, must be previously driven off, and correspondingly small amounts of reagent used.[1]

Exercise 26. Potassium and Sodium

1. *Potassium Chloroplatinate.* Two milligrams of a 1 per cent potassium (chloride) solution are placed on a slide next to a drop of concentrated platinum chloride solution and the drops brought together with a glass thread. Next to the fine-grained turbidity which forms at the point of contact, one can soon see large crystals, deep yellow octahedra and their distorted forms, e.g., six-sided plates. It is possible to obtain a good reaction with as little as 0.02 γ potassium on diluting the test solution. If rubidium and caesium as well as ammonia (laboratory air!) are excluded, the reaction is definite.

Because of the sensitivity of the reaction and because of the easy contamination of the reagent by the laboratory air and glass, the platinum chloride must be tested. "A reagent which gives only an amorphous brown film on evaporation and does not leave any octahedral crystals is actually not obtainable"; it is necessary then to work with quartz vessels and acids purified as carefully as possible. But by comparison one can easily determine whether there are traces of impurities in the platinum chloride or potassium in the sample.

(If the presence of rubidium or caesium in addition to potassium is suspected, the addition of a strong hydrochloric acid solution of gold chloride which is saturated with silver chloride will permit distinguishing between the three. This solution gives no reaction with potassium salts, red prisms with rubidium and black crosses with caesium.)

Rosenthaler (private communication) uses copper lead nitrite mixture in place of the expensive platinum chloride with possibly silico-tungstic acid for differentiation between potassium and ammonium.

2. *Sodium* is identified as *sodium uranyl acetate*, $NaUO_2(C_2H_3O_2)_3$. About 2 mg. of a solution of 1 per cent neutral or better weak acetic acid sodium (chloride) solution are treated with powdered ammonium uranyl acetate, according to Schoorl and Lenz. Pale yellow tetrahedra (with green fluorescence) are obtained, the appearance of which are indicative of the presence of sodium. If uranyl ammonium acetate is unobtainable, a uranyl acetate *solution* acidified with acetic acid which is applied to the evaporated sample may be used. It is, of course,

[1] W. Böttger, Qualit. Analyse, Leipzig, 1925, pp. 465 f.

understood that both these reagents must be free from sodium, i.e., the water solution must leave no octahedra on evaporation.

3. If potassium and sodium are to be detected in the presence of one another, the following procedure is used:

(*a*) The *chloroplatinate reaction* of the potassium is *not* disturbed by the presence of sodium. With sufficient excess of reagent, the octahedra K_2PtCl_6 are first obtained, then the triclinic plates of the sodium compound (Fig. 2, p. 10), and both ions can be very nicely identified in *one* sample especially when the chlorides are used. The test is carried out with about 20 γ of a NaCl and KCl mixture.

(*b*) Detection of sodium as *pyroantimoniate* according to Böttger.[1] A small amount of the solution to be tested for sodium, which must not react acid, is evaporated to dryness; a hardly visible kernel therefrom is placed on the slide and a droplet of the reagent to be described added. The size of the droplet depends upon the amount of the solid residue. In consideration of the solubility relations, only so much reagent is added that supersaturation is reached. With 1 γ Na a definite crystallization is obtained except when about one hundred times as much potassium (chloride mixture) is present.—The form of the crystals depends on the degree of supersaturation: a solution prepared from a large-kerneled potassium antimoniate gives chiefly the (more stable) octahedra, whereas by the use of flourly potassium antimoniate, chiefly sawbuck, cigar- or whetstone-shaped forms appear.

Reagent: About 0.05 g. floury potassium antimoniate is warmed with 5 cc. water for 2 to 3 minutes with frequent shaking to about 50° C. or boiled for several minutes if coarse-kerneled antimoniate is used.

(*c*) Finally, if traces of potassium are sought in the presence of much sodium, Macallum's reagent is recommended, i.e., a mixture of cobalt nitrate, sodium nitrite and acetic acid.[2]

Exercise 27. Ammonium

About 2 mg. of a 1 per cent ammonium chloride solution are placed on the bottom of a gas chamber (p. 24). On the inner side of the cover is placed the ammonium reagent, perhaps platinum chloride in the first experiment, Nessler's reagent in the second and mercurous nitrate (less sensitive) in the third. When everything is ready, the ammonium chloride solution is treated with a droplet of alkali and the chamber closed. The effect is visible in a few minutes: octahedra of ammonium chloroplatinate, brown coloration of the Nessler solution, blackening of the mercurous salt. — For the application of litmus paper, see the organic part (p. 116).

[1] Mikrochem., Pregl-Festschrift, p. 14 (1929).

[2] Molisch, Mikrochemie der Pflanze, 2nd ed., p. 62. The recipe is as follows: 20 g. $Co(NO_3)_2$, 35 g. $NaNO_2$, 10 cc. acetic acid diluted to 75 cc. Allowed to stand until completely clear, kept tightly sealed.

Exercise 28. Analyses

Inorganic Anions

The manner of testing small amounts of solutions for anions should present no difficulties to those who have conscientiously carried out the exercises up to now, since it concerns in most cases only a suitable corresponding application of the macromethods. We will content ourselves therefore with a few selected exercises.

Exercise 29. Sulfates

1. About 1 mg. 1 : 1000 sulfuric acid is mixed in a capillary tube (p. 27) with dilute barium chloride solution. A turbidity or a centrifuged precipitate is visible even with the naked eye. In some cases, gypsum is preferable as form of identification; for this, calcium acetate is used as reagent.—It is to be noted that the sulfur content of the illuminating gas may easily introduce small amounts of sulfuric acid into the test liquid. See also p. 118.

2. A kernel of barium sulfate is reduced to the *sulfide* according to Denigès. A kernel held on the platinum wire is reduced in the upper third of an alcohol flame and then placed in a droplet of sodium nitroprusside solution, the blue-violet color showing the presence of sulfur. If the barium ion is also to be detected in the solution, 10 per cent iodic acid solution is used as reagent, according to Denigès, whereby the quite characteristic bundles (mostly crooked) needles of barium iodate appear.

Exercise 30. Phosphates

Ammonium magnesium phosphate is the most common and best form of identification for *ortho-phosphoric acid*, as already discussed under magnesium. A kernel of magnesium acetate is accordingly placed in the test drop which has been treated with ammonia (and possibly ammonium chloride). The cubic yellow crystals of ammonium phosphomolybdate should also be observed under the microscope at this time. They are prepared by dissolving a kernel of ammonium molybdate in nitric acid of 1.1 specific gravity and the drop brought in contact with a drop of sodium phosphate solution. Octahedra, also rhombododecahedrons, which collect to form spheres, crosses and other forms, appear. For spot reactions see the proper section.

Exercise 31. Fluorides

1. The detection is carried out by transformation into fluosilicate and sodium silicofluoride. Some fluorspar is mixed with concentrated sulfuric acid in a platinum crucible, finely powdered silicic acid is scattered over it and the whole warmed. Inside the cover of the crucible is a small drop, on the outside, a large drop of water. The latter serves for cooling; the former contains some sodium chloride. If the salt

solution is allowed to evaporate on a varnished slide after the action is complete, the crystals of *sodium fluosilicate* appear, pale red, six-sided prisms or plates, also rosettes and stars which must be looked for with small iris diaphragm opening because of the proximity of the indices of refraction (objective protection, p. 6).

An etching test is to be recommended only with the use of larger amounts of fluoride.

2. *Detection According to Feigl and Krumholz.* The apparatus shown in Fig. 66 is used.[1]

The SiF_4 evolved in the glass shell by the sample, quartz sand [2] and concentrated sulfuric acid is caught by the drop of water on the thorn of the stopper and hydrolyzed to silicic and fluosilicic acid. It is warmed for a minute and then allowed to stand for 3 to 5 minutes. The drop hanging on the thorn of the stopper is rinsed into a micro crucible and the solution tested as described below (Exercise 33). Limit of identification is 1 γ fluorine. For practice purposes, minerals containing little fluorine such as zinc blende are recommended. In mineral waters a content of 0.0001 per cent fluorine could be found, using several cubic centimeters. Details are given in the original paper.

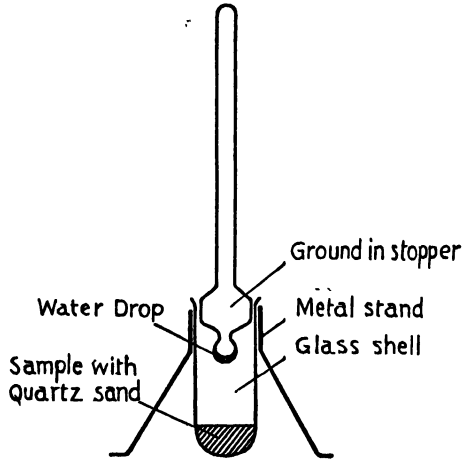


FIG. 66.—Apparatus for detection of fluorides. (App. ¾ natural size.)

Exercise 32. Carbonates

1. The evolution of a gas which takes place upon addition of strong acids is determined.

If the sample is to be tested under the microscope, the carbonate, e.g., a kernel of calcite, is placed in a drop of water and covered with a cover glass. Then it is brought into focus. On the left edge of the cover glass is placed a drop of hydrochloric acid, on the right side a piece of filter paper. As soon as the hydrochloric acid reaches the object the evolution of gas becomes visible.

[1] Mikrochemie, Pregl-Festschrift, p. 77 (1929). Obtainable from P. Haack, Vienna, IX, Garelligasse.

[2] According to Feigl and Krumholz, sea sand may contain fluorine.

Should it be desired to test with limewater in the case of very small amounts, the procedure in Exercise 39 is to be followed with the necessary changes.

2. Since the limewater reaction, because of its sensitivity, may under certain conditions give rise to an illusion, Feigl and Krumholz [1] use a mixture of 1 cc. 0.1 *N* Na_2CO_3 with 2 cc. 0.5 per cent phenolphthalein and 10 cc. water. A drop of this solution is placed on the thorn of the apparatus, Fig. 63. Four γ carbonic acid cause decoloration of the drop. For further details, e.g., detection of carbonates in the presence of sulfides, sulfites and cyanides and detection of oxalic acid by transformation into carbonic acid, see the original paper.

Exercise 33. Silicates

1. *Decomposition with Hydrochloric Acid.* A few milligrams of finely powdered blast-furnace slag are heated in a platinum spoon on the water bath until no grating noise is heard on stirring with the platinum wire. It is then moistened several times with hydrochloric acid, brought to dryness each time and finally extracted with dilute hydrochloric acid. The cations are sought in the solution according to known methods. The residue is silicic acid, which (as above under fluorine) can be identified. If the experiment is repeated with very small amounts, e.g., several γ, the silicic acid may be colored with *malachite green* (solvent: water), *Nile blue* (alcohol), or *methylene blue* (very dilute acetic acid). The dyes are especially suited for making visible traces of silicic acid which remain on the slide on evaporation. The dye solution is placed on it, allowed to stand for a while with gentle warming and then rinsed off with the proper solvent.

2. *Decomposition with Hydrofluoric Acid.* Either pure commercial hydrofluoric acid or a mixture of ammonium fluoride and sulfuric acid is used. The examination for K, Na and Ca according to the procedure of Hemmes will be taken as example: several milligrams of very finely powdered glass are mixed with ammonium fluoride and water in the platinum spoon, a drop of concentrated sulfuric acid added and the whole heated (not to red heat) until no more sulfuric acid fumes are evolved. The residue is extracted with hot water. A drop of the solution is allowed to evaporate; gypsum crystals show the presence of *calcium.* The rest of the solution is evaporated in the platinum spoon, ignited and extracted with water. One part of the solution is evaporated according to p. 84 on a small spot on the slide and tested for *potassium* with platinum chloride. If only a little potassium is found, the rest may be tested immediately for *sodium* with uranyl acetate (p. 103). If much potassium is present, it is precipitated with tartaric acid and the excess reagent removed with ammonium acetate. The

[1] Mikrochemie **8**, 131 (1930).

solution is evaporated, ignited weakly and the test with the uranyl salt repeated.

3. A very good method for the decomposition of silicates is the heating with *lead oxide* according to Canaval. Of course, it may be used only if the presence or absence of lead is established in another way. The sample, which has been stirred together with lead oxide, is fused on a graphite base, then fumed off with sulfuric acid almost to dryness, the residue taken up in water and tested further. Canaval uses this procedure principally for the examination for K, Na, Ca and Mg, but also for Al.

4. Benzidine reaction of silicic acid of Feigl and Krumholz. The drop hanging from the thorn as obtained in Exercise 31 is rinsed into a micro porcelain crucible and 1 drop of molybdate reagent added. This reagent is prepared by mixing 5 per cent ammonium molybdate solution with one-third its volume of concentrated nitric acid. The test is warmed for a *short* time with the microburner, *but not boiled.* After cooling, one drop of a solution of 0.25 g. benzidine in 100 cc. 10 per cent acetic acid and several drops of a saturated solution of sodium acetate are added. A blue coloration shows the presence of fluorine. The limit of identification is 0.1 γ of fluorine. The test may be carried out also on spot test paper, but the limit of identification is then only 1 γ. A blank test with a drop of water should always be carried out when the crucible is used to make sure that the silicic acid is not from that source.

5. On the decomposition of silicates by the usual method of fusion with alkali carbonates nothing further need be said.

Exercise 34. Chlorides, Bromides, Iodides

Since the identification of the anions presents no difficulties if chloride, bromide or iodide ions are present alone, only the case where all three ions may be present is considered.

A simple method depends on the fact that oxidizing agents first liberate iodine and then bromine. Two to four milligrams of a solution containing about 1 per cent of each halogen ion are used and a little starch added by dipping a glass thread into the starch powder and then stirring it up with the solution. A kernel of potassium chlorate is then added, the drop acidified with hydrochloric acid and quickly covered with a watch glass; then the immediate neighborhood of the chlorate is observed, as it is here that the changes start. First a blue color appears which indicates *iodine*; after a time it fades (oxidation to iodic acid) and gives place to the red brown color which shows the presence of *bromine.* The procedure is applicable only when sufficient bromine is present, e.g., it works well with the ratio Br : I = 1 : 1. If smaller amounts of bromide are sought, the iodine must be removed previously, as described below.

Finally, in order to detect *chlorine*, the iodine is precipitated with palladium solution in the usual way, the solution treated with potassium dichromate and evaporated to dryness on the slide. For further treatment the gas chamber, p. 24, is used. The salt residue (containing chromate) is mixed on the lower

slide with some concentrated sulfuric acid, and a drop of sodium carbonate solution is placed on the upper slide. Then the mixture is gently warmed for a quarter of an hour. In the presence of chlorides the sodium carbonate will contain after this time, as the result of the known reaction, some chromate ion, which is identified by means of silver nitrate or diphenyl carbazide. (See p. 100).

At this point it would be fitting to insert an experiment on the detection (and approximate determination) of *traces of iodine*. Since we have not carried out any work in this field at this Institute we believe that we can content ourselves with reference to v. Fellenberg, "Das Vorkommen, der Kreislauf und der Stoffwechsel des Iods," Munich, 1926. An experiment, e.g., with 100 g. of ordinary table salt appears to the author to be suitable.[1]

Exercise 35. Sulfides

1. If a water-insoluble sulfide is present which dissolves in acids, the hydrogen sulfide evolved by the dissolving is recognized by its odor (limit of identification 0.2 γ) or identified perhaps in the gas chamber with a lead oxide thread.[1] The transformation of the sulfur into gypsum is also sensitive and characteristic. The sulfide, such as some powdered zinc blende, is moistened on the slide with some calcium chloride solution and then the slide is inverted over a bottle filled with bromine or bromine water so that the drop is exposed to the vapors of the halogen. In this way the *precipitated sulfides* (and free sulfur) are rapidly oxidized. The *natural* sulfides (glances, pyrites, blendes) mostly form abundant gypsum crystals after 5 to 10 minutes. Molybdenum glance is slowly attacked; the action may be hastened by roasting the mineral for a moment before exposing to the bromine vapors. Of course, the natural sulfides must be used in a very finely divided state.

2. For the iodoazide reaction of F. Feigl, see Exercise 84.

3. If the acidity of the vapors which arise from the roasting of sulfur or sulfides is to be determined, a tube open at both ends and bent in an angle of 135° is used. One arm is held horizontal while the other points upward. The sample is placed at the bend, and a thread of moist blue litmus silk[2] is inserted at open upper end.

Exercise 36. Nitrates and Nitrites

1. *Nitric acid* may first be identified by means of *nitron* (Diphenyleneanilodihydrotriazol).

Nitron forms a precipitate of fine bundles of needles with nitrates.

[1] See also Lunde and coworkers, Mikrochemie, Pregl-Festschrift, p. 272 (1929).
[1] The addition here of a little kernel of zinc to the sample may be advisable.
[2] Feigl: private communication.

Several kernels of the reagent are placed in a 5 per cent potassium nitrate solution mixed with acetic acid and the precipitate recrystallized from a large drop of hot water.

It is to be noted that other acids, such as nitrous, chloric and perchloric, hydriodic (also salicylic, oxalic, ferrocyanic and picric acids), give difficultly soluble nitron compounds, but the precipitates differ in form from the nitrate, which, as said, forms long fine needles.

Sulfuric, hydrochloric, boric and phosphoric (also formic, acetic, tartaric, citric and benzoic) acids form easily soluble nitron compounds.

2. The extremely sensitive reaction which occurs when nitric acid is mixed with a solution of *diphenylamine* in concentrated sulfuric acid is, as known, decisive only when other oxidizing agents (such as nitrous acid, ferric chloride and many others) are excluded. For carrying out the test, a hardly visible particle of dry sodium nitrate on the slide is touched with a small drop of diphenylamine sulfuric acid reagent. Rosenthaler [1] combines the nitron reaction with the diphenyl-amine reaction, the solution of nitron (1 g. in 10 cc. 50 per cent acetic acid) being allowed to act first, whereby the precipitation of needles may take place. The preparation is allowed to dry up and is then touched with the diphenyl-amine reagent, the crystal bundles forming blue islands. One can proceed similarly with the Rupe reagent (α-dinapthomethylamine).

3. If the reaction with ferrous sulfate, which makes up in reliability what it lacks in sensitivity, is to be employed, the sample to be tested is evaporated likewise to dryness. Then it is moistened with concentrated sulfuric acid, and a kernel of the ferrous salt is added by means of the platinum needle. The microscope is focused on this kernel (condenser and low magnification!) A beautiful reaction may be obtained even with only a few micrograms. It is obvious that one must test for nitrous acid by a separate experiment.

A trace of hydrochloric acid (chloride) should be added to the substance to be tested in the ferrosulfate as well as in the diphenyl-amine test.

4. Nitrous acid is detected by the addition of several starch grains, potassium iodide and a trace of dilute sulfuric acid. Since nitrates can be converted to nitrites by reduction, e.g., by zinc in acid solution, electrolytically or by heating in some cases, this reaction offers the possibility of identifying nitric acid in conjunction with one of the above-mentioned reactions.

Exercise 37. Insoluble Residues

In chemical investigations of mixtures, *residues* which are insoluble in most acids are often obtained. Since the quantities of such insoluble remainders are often not sufficient for examination by the usual methods, microanalysis may be of great service in such cases. Schoorl recommends that the following preliminary tests be made on such residues. (For a practice preparation, a mixture of sulfur, gypsum, lead

[1] Pharmaz. Ztg. No. 5 (1929).

sulfate and others of the substances mentioned below may be used. The following procedure may be carried through with a few milligrams of this mixture.)

1. The sample is heated over the microburner to see if a *sublimate* forms. The sublimate may be sulfur and is tested as described above.

2. The heated residue is boiled with a large drop of *water*, filtered if necessary, some acetic acid is added and the drop allowed to evaporate. On sorting through the residue, *gypsum* crystals may be found. It may be mentioned at this point, however, that traces of this substance are often met with in microchemical work (sulfur content of illuminating gas, calcium content of the glass and acids), and on the other hand that gypsum is seldom found in a residue which has been repeatedly treated with acids. Finally, the water extract may also contain *lead sulfate*, the solubility of which (1 : 20,000) is sufficient to permit testing by means of the triple nitrite reaction.

3. The residue remaining from the foregoing tests is treated with hot *concentrated sulfuric* acid. *Barium sulfate*, *strontium sulfate* and *lead sulfate* may crystallize out of this solution.

As a rule, barium sulfate forms small crosses which may be compared to two whetstones which penetrate each other at 90°. Strontium sulfate is similar; the crystals are usually larger than the barium salt and beside the crosses there are often also rhomboidal plates. Make a comparison preparation.

4. When the excess sulfuric acid is driven off and the extraction with water repeated, *silver sulfate* will go into solution if the original residue contained *silver halides*. The treatment with sulfuric acid also serves to prepare the oxides of tin, antimony, iron, aluminum and chromium for later treatment.

5. In order to confirm the presence of *lead sulfate*, the sample is extracted with ammonium acetate. The sample is warmed with a drop of the concentrated solution, diluted with water and drawn off. Copper acetate is added, the drop evaporated and the residue tested by means of the triple nitrite reaction or by conversion into lead iodide according to p. 85.

6. The residue from 5 is extracted with nitric acid whereby sufficient amounts of *iron, aluminum and chromium oxides* are dissolved to permit separation and identification.

7. After extraction with concentrated hydrochloric acid, the solution is tested for *tin* with rubidium chloride and for *antimony* with caesium chloride and potassium iodide.

8. The residue from the last treatment should contain essentially

only silicic acid and *silicates*, which are decomposed according to known methods.

9. *Calcium fluoride* is tested for in a separate portion with precipitated silicic acid and sulfuric acid.

The results of this preliminary testing will determine whether a decomposition by fusion with potassium carbonate or by heating with ammonium fluoride and concentrated sulfuric acid should be carried out. If there is reason to assume the presence of difficultly soluble halogen compounds, an extraction with sodium thiosulfate may be necessary.

Exercise 38. Analyses

Organic Part

Qualitative Elementary Analysis

Exercise 39. Carbon

1. Since, as is known, not all organic substances carbonize on the slide or platinum foil, a sample is always heated also in a tube sealed completely or at one end. Difficultly fusible tubes of ½- to 1-mm. bore are used. Liquids are allowed to enter through a drawn-out point; solid materials are introduced with a glass thread. The section of the tube *above* the sample is heated first, then the sample itself, so that the vapors must pass the glowing spot. The carbon appears usually as a shining mirror, which proves to be combustible on opening the tube. Experiment with about 10 γ anthracene and 10 γ chloroform.

2. If very small amounts are to be detected with the greatest degree of certainty, the substance is burned in a sealed tube with *oxygen* and the carbon dioxide detected as calcium carbonate.

The piece *AB* (Fig. 67) of approximately the same shape and size as in the illustration is drawn from a difficultly fusible glass tube about 5 mm. in (outer) diameter which has been thoroughly ignited throughout its whole length just previously. It may also be drawn from a wide combustion tube. It is sealed at both ends and reserved for use. Before carrying out a reaction, *AB* is cut in two in the center at *a*. Thereby two "combustion tubes" are formed. One is drawn out further so that a piece α, a few centimeters in length and only 0.3 mm. in thickness, is formed. Then a short piece of heavy rubber tubing K_1 which is so measured as to form a perfect connection with the tube *MNP* is pushed over the combustion tube in the direction from α toward γ.

MNP is a T-tube, as can be seen. It is held in a retort clamp (not shown). The arm *P* is vertical, and the part *MN* horizontal. The second opening may be closed by a cork K_2; the third is connected with a soda lime tube, a fixed alkali

wash bottle and an *oxygen* gasometer, in that order. This apparatus is of course joined glass to glass by short pieces of tubing. The stopcock of the gasometer

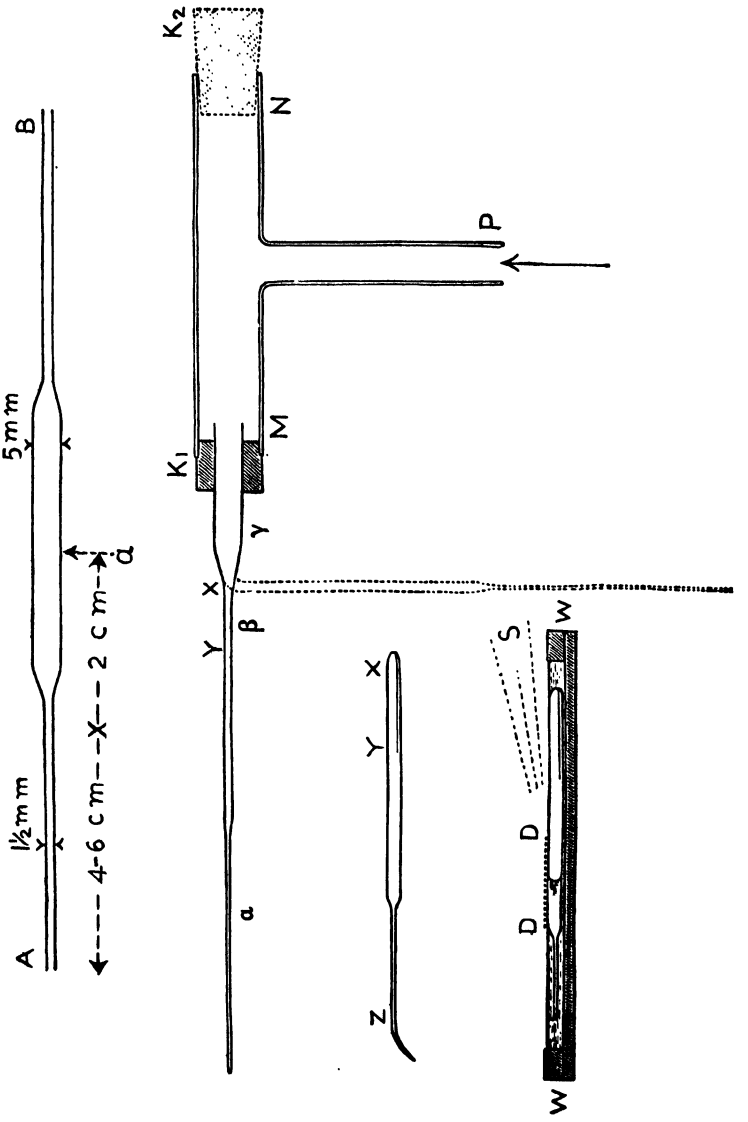


FIG. 67.—Detection of carbon.

is so set that, with stopper K_2 opened, a stream of gas emerges at the rate of about two bubbles per second.

In order to be certain, we ignite the tube $\alpha\beta\gamma$ once more by stroking it along

its length with the microflame. In order that the tube does not change its form too much during this operation it is allowed to fall away from the point x so that it makes a right angle. (See the dotted position.) Then the combustion tube is drawn out fine at α and thereby sealed and at the same time bent a trifle so that it may be opened conveniently later. During all these operations, the cork K_2 is closed, so that the stream of oxygen must pass through the tube. The latter is then bent straight again at x (for which the slight excess pressure in the interior produced by the gasometer is very convenient and helpful).

The substance is now introduced. This can be done in a variety of ways, depending upon the nature of the substance in question. In the case of difficultly volatile liquids or of a powder, a trace is placed on the end of a freshly drawn glass thread from 0.15 to 0.30 mm. thick. A little groove can also be made of platinum foil 2 mm. long and not quite 1 mm. wide, fused on the end of the thread and the substance placed upon it. If very little substance is available, the latter manipulation is best carried out under the binocular microscope. *Since every touch of the fingers must be avoided*, platinum forceps and a platinum needle which have been ignited directly before are used.

Volatile liquids or also powders may be conveniently taken up on a freshly drawn out narrow glass tube 0.1 to 0.3 mm. in diameter. One must guard against an excess especially with the liquids! The sample is introduced in all cases by pushing in the glass thread or tube mentioned above through the wide part of the T-tube up to the point y after removing the cork K_2. During this operation the oxygen goes out through N and thereby hinders the entrance of air containing carbon dioxide into the combustion tube. Then the tube is fused off at x, leaving the part of the glass thread in the tube.

If the substance contains halogen or sulfur, some lead chromate is introduced into the tube. This is powdered, ignited and then either brought on the above-mentioned platinum groove together with the substance or caught up by means of a special tube 0.5 mm. in diameter and introduced into the combustion tube like the substance. In this case, the use of a stopper of platinum foil is also recommended so that the particles of the lead chromate do not later fall into the limewater. The combustion tube may be examined under the microscope, e.g., to ascertain whether the substance has not been lost during introduction.

In order to carry out the *combustion*, the tube is placed in an ordinary combustion tube open at both ends, 25 cm. in length and 1 cm. in width, one end of which may be closed with a cork, the other remaining open. This tube is heated in a horizontal position directly under the little combustion tube, *being rotated continuously*. For this purpose one or two strong Bunsen burners are used, and the tube is heated slowly until the glass colors the flame and then for 1 minute longer. Under these conditions the little combustion tube does not adhere to the wider tube, and also it will never be blown up at one side; at the most, it enlarges its volume slightly, but uniformly. This enlargement is favorable for one of the following manipulations. (No explosion of the tube occurred during hundreds of experiments.)

Then the combustion tube is allowed to slide out of the wider tube (without waiting for it to cool off, perhaps on an asbestos plate), and it is placed in a small

vial half filled with clear limewater with the tip *z* (Fig. 67) downwards, where the tip is broken off with a strong forceps or by pressing it against the bottom of the vial. The limewater rises in the wide part of the ignition tube; should this not occur in an exceptional case, part of the (gaseous) contents are forced out, e.g., by heating. On cooling, some limewater will enter. Then the broken-off tip is sealed again. This may be done in various ways, e.g., by means of a piece of sealing-wax or by allowing some vaseline or molten (anhydrous) lanolin to enter and then fusing shut with the microflame. (If this is not done, unpleasant spattering of the limewater occurs.) A piece of evidence of unlimited keeping quality is thereby obtained.

The concluding step is the examination of the inclosed limewater. With larger amounts of substance, such as one microgram, there appears on the meniscus of the liquid a decided turbidity which is visible with the naked eye and which penetrates the reagent to a certain depth in a characteristic cloudy manner. With smaller amounts of substance a magnifier or microscope is used for the examination. A dark background is used; the sample is best illuminated by a micro arc lamp so that the cone of rays (Fig. 67) is directed to the surface of the limewater. If the examination was to be carried out particularly carefully, the author placed the ignition tube in a cell of water. A cover glass may be placed at *DD*. The carbonate precipitate, fine as dust at first, forms larger rhombohedral crystals and spherical clusters after a time. It should be remarked further that small droplets are often seen in the neighborhood of the limewater meniscus on the wall of the ignition tube, by the incidence of the cone of rays; they are due to condensation and cannot be confused with a precipitate if carefully observed.

In practicing, blank experiments, e.g., with ignited silicic acid or the like, are to be carried out; *their result must be absolutely negative.*

What substance is to be used for practice is, of course, entirely immaterial; we recommend perhaps a solid sample (cane sugar), a liquid (chloroform) and several cubic millimeters of illuminating gas which are introduced into the apparatus in a capillary. Of the solid sample, several small particles are to be taken, as small as can still be seen with the naked eye, i.e., a few γ. For this amount, the oxygen filling of the combustion tube is also sufficient. If too much substance is taken, the tube often appears lined with a black mirror of carbon after the ignition. The carbon is thereby also identified, but the experiment loses in cleanliness.

The limit of identification is within several millionths milligram.

A simple experiment, which demonstrates the sensitivity, consists of drawing between the fingers the above-mentioned glass thread used for the introduction of the substance. A very definite reaction is obtained, and it is entirely immaterial whether the hands have been previously washed with soap or not. The result is the same if the thread is drawn through the hair or the mouth. The experiment is slightly reminiscent of a generally known experiment in flame coloration.

The procedure is obviously also applicable to problems of *inorganic chemistry*. *Graphite* burns rapidly enough to give a strong reaction after one minute of igni-

tion. A piece of florist's wire (7 mg. in weight), sandpapered and briefly ignited, gives a strong reaction. *Diamond* powder burns if it is well heated in a quartz tube with the blowpipe.

In experiments in which the substance does not disappear on ignition, it will contaminate the limewater and interfere with the test. Therefore, a stopper made by crumpling together a piece of platinum foil 4 by 4 mm., is placed at γ (Fig. 67). The foil is particularly well ignited since otherwise the limewater will infallibly appear turbid.

3. A shortened procedure is the following: a combustion tube as described above is prepared and filled with air practically free from carbon dioxide by sucking at the wider end with the mouth while holding the narrower end in the air space above the solution in a bottle half filled with alkali. After this the point is sealed, the substance introduced without the use of the T-tube and finally the wide end also sealed. Lead chromate which may be necessary is introduced before sucking in the air. The other manipulations are the same as with the exact procedure.

For many cases this method of working will suffice. But it must be mentioned expressly that the blank test will show a trace of carbonate. If one considers on the other hand that 10 cu. mm. of ordinary atmospheric air contain only 0.004 γ CO_2, corresponding to 0.001 γ carbon, it is clear that with 1 γ of substance one cannot make a false decision.

Exercise 40. Nitrogen

1. *Lassaigne Test.* Five to ten γ urea are kneaded together in a capillary tube 1 mm. wide with some metallic sodium by means of a wire. The tube is then heated from the top to the bottom, and the test for cyanide ion carried out. The crushed tube is brought together with water and some ferric sulfate in a micro test tube, heated and centrifuged. The clear solution is drawn off and acidified with hydrochloric acid. The flakes of Prussian blue often appear only after a time.

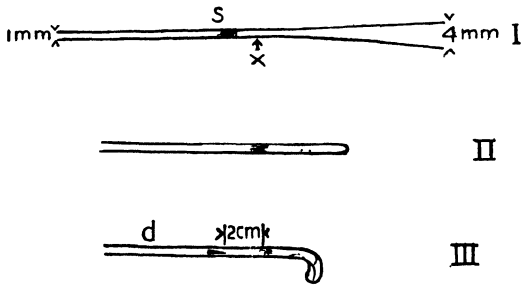


Fig. 68.—Detection of nitrogen.

2. The conversion of the nitrogen into ammonia is more sensitive. A tube, I, Fig. 68, is fitted with an asbestos plug which is ignited in the platinum forceps, trimmed, and then pushed into the tube from the wide end. By heating the place where it rests until the glass softens it will adhere well and fill out the cross-section. Then some air-slaked lime is placed at the point x, after this the substance, e.g., 0.5 γ urea, finally again lime. The entire heap may amount to a few milligrams.

The substance may be introduced also as solution by means of a capillary tube. The mixing is accomplished by means of a platinum wire or by tapping. Then a piece of hardened filter paper is cut in the form of a long isosceles triangle, the base of which corresponds to the width of the tube and whose height is 4 to 5 mm. The point is dipped for a moment in red litmus solution so that this spreads at the most over a length of 1 mm. Then this reagent paper is pushed with the point foremost into the tube to within 1.5 to 2 cm. of the asbestos plug. The tube is examined under the microscope to ascertain whether the reagent paper is faultless. The heating of the substance-lime mixture is carried out after this as follows: the tube is sealed at the right end (II), and then *that* part of the mixture nearest the asbestos plug is heated. After this, the Bunsen burner, of which only the pilot flame has been used up to this point, is turned so that the flame is 1 or 2 cm. in height, and the part of the tube containing the mixture (III) is allowed to collapse. In this way all the ammonia is forced into the space in front of the plug. The litmus solution is prepared by adding sulfuric acid to a concentrated blue litmus tincture until the hydrogen ion concentration of the solution is 0.1 to 0.05 *N*. Thereby one obtains, to be sure, a somewhat lower sensitivity but avoids deceptions which may (by the use of sensitive red paper) occur as a result of the alkaline reaction of the glass. Good results are obtained also with mercurous nitrate, and if one works with artificial light, this is preferred to the litmus.

The reaction is still successful with 0.1 γ nitrogen, that is, e.g., with 0.2 γ urea. With such small amounts it is recommended that before heating the tubes be evacuated with the water pump and then sealed at *d*.

Considering the sensitivity, corresponding care is necessary. The lime must be tested of course by a blank experiment, and too large amounts of substance must not be used as otherwise traces of impurities may give the illusion of a nitrogen content. As a rule, *several thousandths milligram* is the correct amount, i.e., a few particles just visible with the naked eye.

3. With substances which may contain nitrogen in combination with oxygen, *copper powder* can be added to the lime (soda-lime) mixture. Experiment with about 1 γ picric acid which is ground in the agate mortar with the lime copper mixture.

Exercise 41. Sulfur

1. One γ thiourea in somewhat moistened state is mixed on the platinum sheet or narrow slide with 4 to 10 times as much soda-chlorate mixture (6 parts of

sodium carbonate and 1 part of potassium chlorate). Then it is heated by means of an *alcohol flame* until no further action is visible, dissolved in water and tested with hydrochloric acid and barium chloride.

2. In order to detect the sulfur content of *illuminating gas*, about $\frac{1}{3}$ mg. potassium nitrate is placed on a cleaned flattened platinum wire and heated so high over the pilot flame that the wire glows darkly. After half a minute the wire is rinsed off on the slide with several milligrams of dilute hydrochloric acid; barium chloride is added, etc.[1] Blank experiment with an alcohol flame.

3. Volatile or difficultly oxidizable substances are best heated with nitric acid in sealed quartz tubes to weak glowing.

Exercise 42. Halogens

Test According to Beilstein.[1] "Into the loop of a platinum wire is placed some pulverized copper oxide which adheres to the wire after brief ignition. Then the substance is scattered or dropped on it and the loop brought in the moderate non-luminous flame of a Bunsen burner, first in the inner and then in the outer zone near the lower edge.

"First the carbon burns and the flame becomes luminous, but immediately afterward the characteristic green or blue color appears. Owing to the extraordinary sensitivity of the reaction, the smallest amounts of substance are sufficient for the detection, with certainty, of the halogens contained therein, and by the longer or shorter duration of the flame color one has an approximate measure of the amount of halogen present."

The experiment may, of course, be carried out with any halogen compound. In order to convince oneself of the sensitivity, a milligram loop (just designed for this purpose) is used. The blank experiment is first made with perhaps pure alcohol; then one uses an alcohol chloroform 100 : 1 mixture or a particle of *meta*-brom benzoic acid, etc. Further details in Emich, "Lehrbuch d. Mikrochemie," Munich, 1926.

Special Reactions and Preparative Experiments

Since, as already stated, the "Manual" does not consist of a collection of reactions but rather a selection of experiments which should show the applicability of the micromethods to the field of organic chemistry, the following will be found

[1] The experiment was also successful in Vienna, Leipzig and Innsbruck. Professors Wegscheider, Klemenc, Böttger and Lindner, who have interested themselves in the matter at my request, are to be heartily thanked. In the case of Viennese illuminating gas, which is very carefully purified, longer heating is necessary. The barium sulfate may be reduced by means of an alcohol flame and tested further by the iodazide reaction (Exercise 84). Professor Böttger recommends for the blank experiment an alcohol burner *without a wick*.

[1] Hans Meyer, Analyse u. Konstitutionsermittlung, Berlin, 1922, p. 249.

still less complete than the foregoing, if an exhaustive collection of the microreactions of any substance is expected. The book of Behrens-Kley, as is known, serves this purpose and is herewith *once and for all* referred to.[1] With the preparative experiments, value is laid upon the fact that the purity of the product is determined by a suitable identification reaction. For this purpose the given amounts must suffice. Of course, the experiments may also be carried out, if necessary, on a larger scale. In this case only very few preparations need be held in stock for the work of the "Manual."

Exercise 43. Ethyl Alcohol

1. *Boiling-point Determination in Capillary Tube.* The procedure is exactly as described on p. 32. The boiling-point will be found to be about 77° C.

2. *Fractionation Experiment.* A mixture of perhaps equal parts of alcohol and water is used and the procedure given on p. 34 followed. The experiment may be repeated with 2 drops of wine.

3. Among the *chemical reactions*, the *iodoform reaction* will be described. It is carried out in the centrifuge cone. Potassium hydroxide is added to the alcohol, warmed, iodine-potassium iodide added to yellow color and finally once more alkali to decoloration. Several drops of the reagents are placed on a slide and the requisite small amounts transferred to the test drop with the platinum stirring hook or a glass thread. Usually only a yellowish powder is to be seen with low magnification; on the other hand, the high-power objective shows yellow hexagons and other forms reminiscent of snow stars. The limit of identification is about 10 to 20 γ alcohol. Working with the slide is not recommended because of the volatility of iodoform. The reader is reminded that the reaction is given not only by many alcohols but also numerous other compounds, such as acetone, acetaldehyde, lactic acid, etc.[1]

Exercise 44. Reactions of Ethyl Amine (Hydrochloride)

1. Saturated potassium hydroxide solution causes the separation of the *free base* in droplet form, the extremely fine contours of the droplets making them easily distinguishable from air bubbles. Use narrow iris diaphragm opening.

2. About 30 γ are treated in centrifuging cone with a drop of alcoholic potassium hydroxide and some chloroform and warmed on the water bath; the familiar *isonitrile reaction* occurs which may be included among the microreactions because of its sensitivity.

3. Conversion into *mustard oil.* The alcoholic solution of the amine is treated with 1 to 2 drops of carbon disulfide, allowed to stand for several hours and then evaporated on the water bath. The residue (ethyl amine ethyl-di-

[1] See also L. Rosenthaler, Nachweis org. Verbindungen (Margosches, Chem. Analyse XIX, XX, Stuttgart, 1923). N. Schoorl, Organ. Analyse, Amsterdam, 1920 and 1921 (Dutch).

[1] See also Hans Meyer, Analyse u. Konstitutionsermittlung, Berlin, 1922, p. 474.

thiocarbamate) is warmed with some ferric chloride, whereby the mustard oil is formed, which can be recognized by its odor. Limit of identification is about 1.5 mg. ethyl amine. Ammonia gives under these conditions ammonium thiocyanate and its reaction with iron.

Exercise 45. Several Reactions of Aldehydes of the Fatty Series

1. For testing the reducing action, either ammoniacal silver solution to which a trace of sodium hydroxide has been added is used, or the aldehyde is warmed with a mixture of quinoline, hydrochloric acid, and potassium ferricyanide, which produces in this case the separation of squares, rectangles and rhombs of quinoline ferrocyanate.

2. A great popularity is enjoyed by the reactions with *dimethyl hydrorescorcin* ("methone"), which may be especially recommended also as beautiful examples for micro sublimation and micro melting-point determinations.[1] For the purposes of the present work it will perhaps be sufficient to carry out the catalytic oxidation of methyl alcohol according to the following procedure, for the development of which the author thanks Dr. H. Alber.[2]

A small droplet of methyl alcohol is placed (e.g., with the 0.5-mg. loop) in a porcelain crucible.[3] A piece of copper wire gauze, 2 by 2 cm., folded twice (or a corresponding ball of copper wire), is heated in the flame of the Bunsen burner to bright red heat, and after brief cooling, when the dark red glow has just disappeared, thrown on the drop in the crucible; a slide with a drop of methone solution is placed quickly over the crucible; the formaldehyde formed by the oxidation of the methyl alcohol causes the formation of formaldimethone, which crystallizes out in fine needles on the slide. The limit of identification is 5 γ (one loop [0.5 mg.] 1 per cent solution still gives individual beautifully formed crystals). The experiment requires but a few moments' time.

3. *Formaldehyde* may finally be also transformed into hexamethylene tetramine: an excess of ammonia is added, the solution warmed and concentrated to the formation of an edge crust, after which potassium ferrocyanide and hydrochloric acid are added. Rhomboidal and hexagonal plates of the ferrocyanide appear. An excess of the hydrochloric acid is to be avoided, otherwise the large rhombs of the hydrochloride will form. Hexamethylene tetramine also gives a useful crystal precipitation with perchloric acid, with glycerine, for example, as

[1] D. Vorländer, Ber. dtsch. chem. Ges. **30**, 1801 (1897); also especially G. Klein and H. Linser, Mikrochem. Pregl-Festschrift, p. 204 (1929), where other references are given.

[2] Not yet published.

[3] E.g., form No. 0.768 of the Staatliche Porzellanmanufaktur, Berlin.

solvent. The reaction with iodine-potassium iodide is recommended by Rosenthaler.[4]

4. For further reactions, such as with meta-diamino benzene hydrochloride, with fuchsin-sulfurous acid, etc., see Emich, "Lehrbuch d. Mikrochemie," Munich, 1926. For the application of para-nitro phenyl hydrazine, see Exercise 59 (benzaldehyde).

Exercise 46. Reactions of Formic and Acetic Acids. Higher Fatty Acids

1. One milligram of formic acid, diluted in twenty times the amount of water, is heated in a centrifuge cone with excess magnesium oxide to neutralize and centrifuged. A drop of the clear solution is placed on the slide, a kernel of cerous nitrate added and allowed to crystallize. Colorless aggregates appear which look like pentagon dodecahedrons and show a black cross between crossed nicols. Spheres are often obtained which show the same optical behavior.

2. A second drop of the magnesium formate solution is treated with mercuric chloride solution and warmed to 70 to 80°: separation of calomel (needles), evolution of gas.

3. Oxidation of ethyl alcohol to acetic acid. Several milligrams of alcohol are heated in one of the apparatus shown in Fig. 33, p. 38, with ten times as much Beckmann mixture (5 g. $K_2Cr_2O_7$, 2.8 g. H_2SO_4, 30 cc. water). The acid reaction of the distillate collecting in the knee is determined by means of a pointed strip of litmus paper 1 mm. wide.

4. *Detection of Acetic Acid as Sodium Uranyl Acetate.* The distillate is about half neutralized with dilute sodium hydroxide solution, according to p. 82, then treated with *uranyl formate* and allowed to crystallize; the formation of sodium uranyl acetate (p. 103) is observed.

Remarks. (*a*) Since uranyl formate is not an article of commerce, a simple method of preparation will be given here: Uranyl nitrate is precipitated with ammonia, the precipitate washed with water, dissolved in formic acid and the solution evaporated; the ammonia content of the preparation does not influence its applicability.

(*b*) The higher fatty acids have not come into consideration up to now. As an appropriate exercise the *sublimation of copper isovaleriate* may be recommended. The work is carried out either in an evacuated centrifuge cone or in a capillary which is similarly connected with the water pump. If one sublimes from slide to slide at ordinary pressure a large part of the salt decomposes.

(*c*) The *saponification of fats* is observed under the microscope. (*α*) Accord-

[4] Pharm. Zeitung 1929: "Ökonom. Arzneimittelprüfung," a comprehensive reference work, the perusal of which is to be warmly recommended.

ing to Molisch:[1] The fat, e.g., a droplet of castor oil, is treated on the slide with a mixture of equal parts of concentrated potassium hydroxide solution and concentrated ammonia, covered with a cover glass and allowed to stand for a long time (possibly several days) in a moist room. The oil droplets change into crystalline aggregates of soap. "Often it appears as though the drop had not undergone any change; only on examination under the polarization microscope it appears that it has changed into a spherical crystal." (*β*) According to Rosenthaler:[2] A highly concentrated alcoholic solution of potassium or sodium hydroxide is used. Two milligrams at the most of the oil are required. As comparison test, a droplet of vaseline oil is taken, which of course is not saponifiable.

Exercise 47. Preparation of Nitroglycerine

Several milligrams of glycerol are treated with six times as much of a mixture (preferably cooled with ice) of equal parts of concentrated pure sulfuric acid and nitric acid, mixed with the stirring hook, and allowed to cool for several minutes. Then it is diluted with water, and the nitroglycerine separates in droplets which can be washed by the use of the centrifuge. The wash water is carefully removed with a fine (fire-polished) capillary (a certain care is required here as the drop of nitroglycerine may easily float on the surface of the water), and the remainder is allowed to dry. For this purpose a piece of calcium chloride the size of a lentil is placed in the centrifuge cone and the latter sealed with a cork (Fig. 69). After several hours the *clear* drop may be taken up in a capillary and exploded by rapid heating behind a protective sheet.[1]

Fig. 69.

Exercise 48. Oxalic Acid

1. *Preparation from Wood.* Four or five pieces of pine-wood sawdust are moistened on the slide with a mixture of concentrated potassium hydroxide and sodium hydroxide and heated over the microburner. The preparation becomes first yellow, then brown, and gas is evolved. It is extracted with dilute acetic acid, centrifuged if necessary, and a kernel of strontium acetate placed in the solution on a slide, whereupon the precipitate mentioned below forms.

2. The calcium salt is important among the difficultly soluble salts:

[1] Molisch, Mikrochemie der Pflanze, 2 Aufl., p. 118.

[2] Apoth.-Ztg. **58**, H. 44, 45, 46 (1920), or Mikrochemie **8**, 72 (1930).

[1] For other glycerine reactions see, e.g., Herzog, Mikro. Unters. d. Seide, Berlin, 1924, p. 117; H. Alber, Mikrochemie **7**, p. 21 (1929).

the marks of identification used in macroanalysis (solubility in hydrochloric acid, difficult solubility in acetic acid) are best used for its recognition, for the crystals, rods and short pyramids (envelope forms), etc., are precipitated very small. The strontium salt is similar, but gives somewhat larger crystals.

3. In separations, e.g., of oxalic from mixtures of oxalic, succinic, malic, citric and tartaric acids, vacuum sublimation will serve very well.[1]

Exercise 49. Reactions of Cyanide Ion

1. For the *Prussian blue reaction* see p. 116.

2. *Thiocyanate Reaction.* Potassium cyanide is treated on the water bath with yellow ammonium sulfide and evaporated. The residue is tested with ferric chloride solution containing a little hydrochloric acid.[1]

3. Detection as *silver cyanide* according to Brunswik. A drop of 1 per cent silver nitrate solution is placed on the cover of the gas chamber (Fig. 15, p. 24) and 0.1 mg. of potassium cyanide on the bottom. The cover is lifted for a moment and the potassium cyanide treated with a droplet of dilute sulfuric acid or with sodium bicarbonate and water. After a while the silver nitrate drop becomes turbid. On microscopic examination, *needles* will be seen which show parallel extinction between crossed nicols. This is important for distinction from the cubic crystals of silver chloride, which remain dark in all positions. If the crystals are not well defined, the drop is allowed to evaporate and the precipitate recrystallized from hot 50 per cent nitric acid in which the silver chloride is hardly soluble. As further distinction from silver chloride, the greater stability toward light is to be emphasized: the chloride becomes blue, then violet and finally black in a short time, silver cyanide brown at the most.

According to Brunswik, the great sensitivity of the reaction may be illustrated in the following experiment: (*a*) If illuminating gas [2] is allowed to flow out against a drop of silver solution, the latter becomes turbid in a short time. The needles may be obtained by recrystallization. (See above.) (*b*) If a 250-cc. flask is blown full of cigarette smoke, a cotton plug pushed into the neck (to keep off tarry products) and this covered with a slide carrying a drop of 1 per cent silver nitrate solution, the drop becomes turbid or crystals are obtained as in (*a*).

Silver cyanide precipitates in blue-green crystals from methylene blue solutions (silver chloride shows the same behavior).

[1] G. Klein, Praktikum der Histochemie, Vienna and Berlin, 1929.

[1] A filtered drop of saliva gives a definite thiocyanate reaction if it is treated with an acidified ferric chloride solution and observed with axial illumination, according to p. 92. See also Mikrochemie **7**, 10 (1929).

[2] Brunswik carried out the experiments in *Vienna*; they are also successful with *Graz* illuminating gas.

4. *Detection with Sodium Carbonate Picric Acid According to Guignard.* A strip of filter paper is dipped into 1 per cent aqueous picric acid solution, dried, dipped in 10 per cent sodium carbonate solution and, if not used immediately, dried again. The golden yellow paper can be preserved. In an atmosphere containing hydrocyanic acid (gas chamber, test tube) it becomes *red orange.* It is still possible to detect 20 to 50 γ hydrogen cyanide if the action is allowed to proceed for 24 hours.

Exercise 50. Experiments with Urea

1. *Preparation from Human Urine.* About 4 cc. of human urine are evaporated in a microbeaker on the water bath with the use of an air stream. The resulting syrup is extracted with alcohol and the alcoholic solution again evaporated to a syrup. After cooling, it is precipitated with *colorless* nitric acid (diluted 1 : 1 with water). The nitrate is cooled and allowed to stand for about 1 hour. The resulting crystal mass is filtered off as *thoroughly as possible* with the apparatus shown in Fig. 26, p. 31, and washed twice with the same nitric acid. The nitrate is stirred up in 0.5 to 1 cc. water and barium carbonate added until it no longer effervesces. The solution is then evaporated to dryness and extracted with absolute alcohol, and this conversion into nitrate and back into urea is repeated. The urea obtained can be freed from the last traces of adhering impurities by dissolving in water and decolorizing with animal charcoal, the product obtained on evaporation of the water being crystallized by precipitating the *absolute* alcohol solution with chloroform and allowed to stand.

2. For testing the purity from time to time, the *melting-point* is determined (possibly in the *microbeaker itself* to avoid loss of material). Thereby a series of melting-points, perhaps 125°, 128°, 130°, 130°, may be obtained. See also footnote 1, p. 32.

The yield will be from 15 to 20 mg. or more with sufficient practice. The yield is determined by weighing the (tared) microbeaker on the ordinary analytical balance.

3. For the *optical* properties, see Exercise 3, p. 80.

4. *Chemical Reactions.* (*a*) Several milligrams are heated above the melting-point in a small test tube. The sample becomes solid and then forms essentially a mixture of cyanuric acid and biuret. The two substances are separated by means of warm water, which dissolves chiefly biuret (testing with copper sulfate and alkali, perhaps, according to p. 92), the residue of cyanuric acid is converted into the copper ammonia salt, i.e., it is dissolved in concentrated ammonia and a drop

of copper sulfate solution added. A light violet precipitate forms, under the microscope almost colorless rhombohedrons.

(*b*) Nitric acid precipitates the difficultly soluble nitrate in the form of monoclinic hexagonal or rhombohedral plates which often overlap like roofing tile. Acute angle 82°. Oxalic acid also gives a well-crystallizing addition compound, but it is more soluble than the nitrate.

(*c*) A crystal of *xanthydrol* is placed in an aqueous solution of urea previously acidified with acetic acid: fine needles of the dixanthyl urea form. The limit of identification is about 0.3 γ urea.

The experiment may be carried out with urine as follows: A droplet of urine (e.g., 1 mg.) is mixed with an equal amount of glacial acetic acid in a capillary, transferred to the slide and powdered xanthydrol placed on the edge of the drop; a finely crystalline precipitate forms mostly around the reagent, and at a greater distance needles and clusters of the dixanthyl urea appear; sometimes they also grow directly out from the reagent. The value of the xanthydrol reaction may be increased by a control experiment: when *urease* is added to the urine solution, it can be seen that the reaction is negative after the decomposition of the urea. (according to experiments of Dr. H. Alber).

Exercise 51. Glucose

Fermentation Experiment. Because of the difference in sensitivity of the tests involved, the detection of carbon dioxide and alcohol is carried out best in separate samples.

(*a*) For the detection of *carbon dioxide*, two tubes (Fig. 70) are used. The grape sugar solution, containing at the most 1 mg. of sugar, and previously treated with a trace of yeast, is sucked into one end of one of the tubes. About 10 cu. mm. of clear limewater are allowed to rise in the other end. The ends are then sealed. A similar blank tube contains only washed yeast suspended in water in place of the sugar solution. After 12 hours the test capillary shows a heavy precipitate of crystalline calcium carbonate; the blank tube, a slight precipitate.

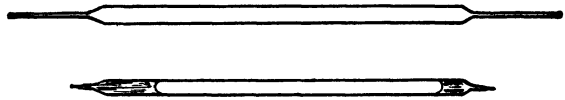


Fig. 70.—For fermentation experiment.

The apparatus shown in Fig. 66, p. 106, might also serve very well for the fermentation experiments.

(*b*) About 20 mg. of sugar dissolved in 0.2 cc. of water and also treated with yeast are used for the detection of the alcohol. The sample is allowed to ferment for 12 hours in the loosely covered microbeaker. After this, the yeast which has not settled out is centrifuged out, and

the solution is transferred into the fractionation apparatus (Fig. 30, p. 34). The first fraction is used for a boiling-point determination, according to p. 32; the iodoform reaction is carried out with the second.

Exercise 52. Preparation of Nitrobenzene

Twenty cubic millimeters of nitration mixture, i.e., a mixture of 4 drops of concentrated sulfuric acid and 3 drops of concentrated nitric acid, are placed in a test tube of 1- to 2-cc. capacity. To this are added 12 cu. mm., i.e., 10 mg., benzene (micropipet, see p. 22). After first stirring for 2 minutes without warming, the test tube is placed halfway in a simmering water bath so that it acquires a temperature of about 60° C. After half an hour, during which time it is occasionally stirred, 0.2 cc. (3 ordinary drops) of water are added, and the mixture (transferred perhaps to a centrifuge cone) is centrifuged, whereby the nitrobenzene sinks to the bottom as a clear drop. It is washed, using the siphon and centrifuge according to p. 26, until the wash water shows a neutral reaction. About four changes of water will bring this about. The nitrobenzene may also be extracted with ether, proceeding as in the case of aniline, although washing with water is recommended in this case also. The nitrobenzene is dried in the centrifuging cone for 5 minutes on the boiling water bath, then a kernel of calcium chloride is placed in the tube as shown in Fig. 69, p. 122, and allowed to stand over night. If these directions are followed, the boiling-point of the product (weight about 5 mg.), determined according to p. 32, will be correct. Should this not be the case, the entire sample obtained is transferred by means of a fine capillary to the tube shown in Fig. 30 (p. 34) and fractionated. Boiling-point: 201–205°; for further characteristics, see Exercises 53 and 56.

Exercise 53. Reduction of Nitrobenzene to Aniline. Reactions of Aniline

Fifty milligrams of tinfoil[1] are added to 20 mg. of pure nitrobenzene in a 3-cc. test tube, and concentrated hydrochloric acid is added in small portions. If the odor of nitrobenzene has not disappeared after a quarter of an hour, the mixture is warmed on the water bath, and, if necessary, more tin and hydrochloric acid are added. Usually the liquid congeals on cooling to a paste of the tin double salt. Concentrated (i.e., saturated in the cold) potassium hydroxide solution is added and the aniline extracted with ether. The extraction is carried

[1] This is weighed out the first time; after that it is estimated by area.

out by pouring 0.5 to 1 cc. ether over the (cold) paste, stirring up thoroughly, centrifuging if necessary, and finally transferring the ether solution into a microbeaker by means of the capillary syphon (p. 26). This is repeated once or twice, and the combined ether extracts are evaporated on the water bath. Yield: usually only 5 to 7 mg. Several properties of aniline:

1. Boiling-point: 184° (corrected, at 760 mm. pressure). The preparation must be dried with caustic alkali; otherwise the procedure is the same as for nitrobenzene.

2. Specific gravity when moist: 1.02, i.e., about the same as that of a 2.8 per cent sodium chloride solution. In order to determine this, 2.8 g. of sodium chloride are dissolved in 98 cc. of water and a coarse emulsion prepared by vigorously stirring 1 to 2 mg. of aniline and a drop of the salt solution in a centrifuge cone. The aniline droplets remain suspended—at least for a short time—and remain so even after brief centrifuging. Addition of several milligrams of water or sodium chloride causes the aniline drops to fall or rise. The procedure constitutes an application of the "suspension method." More detail in Emich, "Lehrbuch d. Mikrochemie," Munich, 1926, p. 105.

3. *Chemical Behavior.* (*a*) On addition of platinum chloride and sodium iodide, individual black crystals and stars of the iodoplatinate appear. The former have usually square or rectangular outlines, but also often pointed ends.

(*b*) A sliver of pine (several cells are sufficient) is colored yellow if it is brought in contact with a solution of aniline in dilute hydrochloric acid. (The toluidines and other homologs also give this reaction.)

(*c*) Bromine water gives a reddish white precipitate of tribromaniline, fine colorless needles under the microscope. Larger crystals can be obtained if a droplet of alcohol is added.

(*d*) Potassium iodide-iodine and sodium sulfate give "aniline herapathite," brownish red rhomboidal plates which do not show any dichroism worth mentioning. The reaction can also be carried out so that the aniline is converted into the sulfate with dilute sulfuric acid, the crystals filtered dry with paper, potassium iodide-iodine added and the precipitate quickly observed.

(*e*) Conversion into acetanilide and symmetrical diphenyl urea, as described in the following exercises.

Exercise 54. Preparation of Acetanilide. Recrystallization in Melting-Point Tubes

A capillary tube (p. 27) is drawn out again at the end in the microflame so that a fine-pointed glass tube is formed, of about 1.5-mm. bore

and 12-cm. length. Three milligrams of glacial acetic acid and 2 mg. of aniline are drawn through the point, both ends of the tube are sealed and it is heated for a quarter of an hour at about 150° either in an ordinary drying oven or a liquid or vapor bath (p. 14). After cooling, the tube is opened at the empty end, and a droplet of water is introduced by means of a capillary. On stirring, crystallization occurs. The crystals are washed in the tube with water, i.e., alternately stirred, centrifuged and the water drawn off with a fine capillary. In order to be able to determine the *melting-point* the crystal mass must first be dried by leading in a stream of dried air while hot. This may be done in a special apparatus[1] or in our case best in the melting-point apparatus itself. This consists, as is known, of a sulfuric acid bath (beaker with stirrer) and a thermometer. Air is led into the tube containing the substance by a capillary (Fig. 71*a*) $\frac{1}{3}$ to $\frac{1}{2}$ mm. in outer diameter, which is bent at *b* at a very obtuse angle so that it acts as a spring and holds the melting-point tube without breaking. In the wide upper part the tube has the dimensions of an ordinary glass tube; it is also bent as shown in the illustration and clamped at *c* by means of a retort clamp which may be on the same stand as the sulfuric acid bath and the thermometer. The end *d* is connected with a sulfuric acid wash bottle and an air gasometer or pump. At *e* is a dust filter (asbestos plug). The melting-point tube is shown shortened in the illustration.

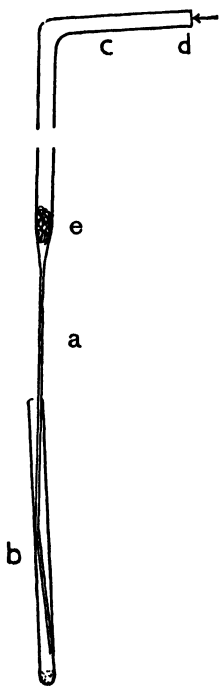


FIG. 71.—Drying substances in melting-point tube.

After the apparatus has been set up, the dried air stream is led over the crystal mass while the temperature of the bath is raised to 100°. The drying continues for 5 minutes at this temperature, the air stream is then shut off and the bath heated gradually to the melting-point of the crystals. Since the preparation is not yet pure, the melting-point will be found to be, e.g., 108° instead of 114 to 115° C. (uncorrected).

For recrystallization, hot (residue-free) benzene is used. About an equal volume of benzene is brought into the melting-point tube by suction or by means of a fine capillary and the solvent centrifuged to the sealed end. The recrystallization is carried out in the *sealed tube*;

[1] See also Aug. Fuchs, Das Schmelzpunktsröhrchen als Reagensglas, Monatsh. Chem. **43**, 129 (1922); W. Friedel, Biochem. Z. **209**, 65 (1929).

the open end is therefore also sealed and the tube placed in an empty test tube which serves as protective tube and air bath. When the test tube is stroked with a Bunsen flame the acetanilide dissolves completely in the benzene; if the solvent condenses in the upper part of the tube it is quickly forced back to the solution by a flip of the hand. The solution crystallizes on cooling, i.e., on the removal of the capillary from the test tube. Should this not happen, one of two things may be the reason. Either the solution is supercooled—in which case it is cooled further by wrapping in some cotton and allowing ether to drip upon it; or too much benzene was used—then the solution must be concentrated somewhat. This is done as described above for drying. In order to obtain a good crystallization, it is advisable then to seal the tube again and recrystallize as before.

In order to separate the crystal mass from the mother liquor, the mass is first pressed together somewhat with a glass rod and centrifuged, and the mother liquor is removed by a fine capillary (which may of course be emptied by blowing out, e.g., on a slide where microreactions may be carried out). The pressing, centrifuging and decanting are repeated several times. After this, the preparation is again dried and the melting-point determined. In order to lose less time, the sulfuric acid bath is allowed to cool only to about 80°. This procedure is repeated (2 or 3 times) until the melting-point is constant. For further check a *mixed melting-point* is carried out: some pure acetanilide is added to the product obtained, mixed, e.g., by careful fusion, and the melting-point determined again. It should not have changed. Further details in Emich, "Lehrbuch d. Mikrochemie," Munich, 1926.

Exercise 55. Symmetrical Diphenyl Urea

Three milligrams of urea are mixed with three times as much aniline in a ball tube *ab* (Fig. 72, I). The tube is sealed at *b*. It is heated for 15 minutes in boiling nitrobenzene. Then the tip is opened at *b* and

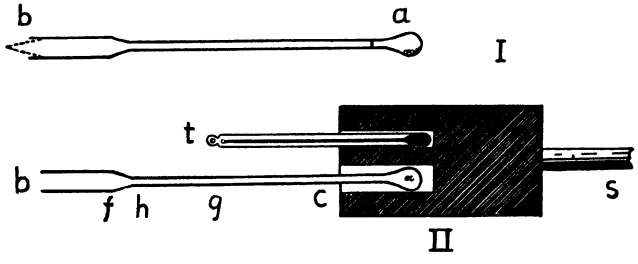


FIG. 72.—Preparation of diphenyl urea.

the reaction product purified in the following way. The excess aniline is first distilled off in vacuum by connecting the suction pump with *b*, heating the tube in a small (Thiele) copper block (Fig. 72, II) and driving the condensing droplets to *f* by means of a microflame. After this the preparation is sublimed in vacuum, the point *g* being cooled by covering with wet filter paper. Then the tube is sealed at *c*, cut off at *h* and the product recrystallized twice from alcohol in the tube. Melting-point: 236°. In distilling off the aniline the temperature is about 120°; in subliming the diphenyl urea, 180 to 220°.

It may be remarked that in the illustration *t* is a short thermometer (Fig. 48, p. 64), and the block, the dimensions of which are about 5 by 3 by 3 cm., is clamped in an ordinary stand by means of the iron rod *s*.

Exercise 56. Conversion of Nitrobenzene into Hydrazobenzene and Benzidine

About 0.1 g. of zinc dust, 5 mg. of nitrobenzene and ten times as much alcoholic potassium hydroxide solution are placed in a sealing flask (Fig. 72, I). In order to obtain thorough mixing on shaking, a piece of zinc the size of a pinhead is added to the zinc dust. The sealing flask is heated on the water bath with frequent shaking. This can be done conveniently by joining the handle of the flask to a somewhat wider tube by a piece of rubber tubing. This second tube projects from the water bath. After 2 hours the flask is opened; this must obviously be done with proper precautions (goggles!) as with all manipulations with sealed vessels with excess internal pressure. The tube is wrapped with a cloth; the tip is scratched and broken off. Then the flask is placed neck downwards in a test tube,[1] centrifuged and the interior rinsed out with alcohol in the same way. The clear solution is drawn off in a second test tube and treated with four times as much water. Impure hydrazobenzene is precipitated. It is centrifuged off and washed with water. The reaction with Fehling's solution (precipitation of Cu_2O) is ascertained on one sample. The remainder is allowed to stand over night with a drop of 3 per cent hydrochloric acid whereby it is converted into benzidine. This solution is now precipitated with dilute sulfuric acid and the crystalline nature of the sulfate determined (needle clusters, plates). Recrystallize from hot water, wash with cold water, dry off with filter paper. Further reactions: (*a*) Does not reduce Fehling's solution. (*b*) Conversion into benzidine blue: a sample is moistened on the slide with 1 per cent potassium dichromate: fine deep blue needles. In the presence of mineral acids, sodium acetate must be added.

It is possible to prepare *pure hydrazobenzene* in this way, but it is recommended that somewhat larger amounts of substance be used; otherwise difficulties will be met with in recrystallization.

[1] The ball of the flask should not lie directly on the mouth of the test tube, as otherwise the tube is easily broken. In such cases a rubber washer or the like is always interposed.

Exercise 57. Phenols and Quinones

The student who has performed the experiments given up to now can easily set up a suitable program for phenols and quinones.[1] We will content ourselves with several hints.

1. Benzene sulfonic acid may easily be prepared by heating benzene with double its volume of fuming sulfuric acid in sealed tubes at about 100°. The procedure is given in Holleman's "Einfache Versuche, usw." (Leipzig, 1916) 60, or in the books cited in footnote 1.

2. The conversion of sulfonic acids into phenols by *potassium hydroxide fusion* may be carried out in a silver or platinum spoon or even a test tube. The melt is acidified with hydrochloric acid and the phenol extracted with ether. (See aniline.)

3. The conversion into tribromphenol is emphasized among the reactions of ordinary phenol. Fine yellowish white needles are formed by exposing an aqueous solution of phenol to the fumes of bromine.

4. Picric acid is distinguished by the refractive indices 1.56 and 1.95. The unusually large difference is to be noticed. See also the instructions in Exercise 3*b*, p. 80. Beautiful guanidine salt, recrystallized from hot water.

5. Instructive reactions may be carried out with the polyhydric phenols. For illustrating their action as *reducing agents*, either neutral or ammoniacal silver solution may be used, depending upon conditions (Rosenthaler, private communication); a trace of alkali is added to the latter solution. Behrens recommends for the same purpose the mixture of potassium ferricyanide, hydrochloric acid and quinoline mentioned under aldehydes (p. 120, 1). We may mention further the conversion of *resorcinol* into *fluorescin* by heating with phthalic anhydride (with the possible addition of some zinc chloride). The test is very sensitive but not exactly characteristic.[2] Phloroglucinol in hydrochloric acid solution colors wood fibers an intensive red, as is known. See also aniline.

6. Numerous microreactions are known for *quinones*. Many quinhydrones show splendid pleochroism. A compound which is formed when chloranil is mixed on the slide with some dimethyl aniline and some benzene acts in the same way. Long flat prismatic crystals showing deep blue-light gray pleochroism. Thick crystals are opaque.[3]

[1] See the known works of Hans Meyer and H. Behrens; also Emich, Lehrbuch d. Mikrochemie, Munich, 1926. For preparative purposes, see perhaps also Emil Fischer's little Anleitung zur Darstellung Organischer Präparate, Braunschweig, 1922. For books in English, see H. L. Fisher, Laboratory Manual of Organic Chemistry, John Wiley & Sons, New York, 1924; R. Adams and J. R. Johnson, Elementary Laboratory Experiments in Organic Chemistry, Macmillan, New York, 1928; Gattermann, Schober and Babasian, Practical Methods of Organic Chemistry, Macmillan, New York, 1925.

[2] Emich, Lehrbuch d. Mikrochemie, Munich, 1926, p. 241.

[3] The preparation (perhaps for demonstration with the projection microscope) must always be freshly made. If imbedded in "Wiesein" the well-known adhesive of microtechnic (obtainable from Th. Schröter, Leipzig-Connewitz), it may be kept for several days.

Exercise 58. Liquid Crystals

1. Several milligrams of (commercial) *benzamide* are first placed on the narrow slide, covered with a cover glass and heated over the microflame until molten. The cover glass is chosen somewhat thicker than usual, e.g., made by cutting up a thin slide. This prevents the preparation from cooling too rapidly. If the preparation is placed quickly between crossed nicols,[1] the melt appears dark, i.e., "isotropic." As it assumes the solid state, the field becomes bright, and more or less vivid interference colors appear, depending upon the thickness of the preparation. By far the largest number of substances behave in the same way, i.e., *the transition from the amorphous liquid to the crystalline solid state follows immediately.*

2. If the experiment is repeated with (commercial) *para*-azoxy anisol, a difference is seen already macroscopically; the melt appearing at first is turbid and becomes clear only on further heating. On cooling, the reverse process takes place. If the solidification is observed under the microscope between crossed nicols, *the turbid melt appears bright*; it is therefore "anisotropic," i.e., it affects polarized light like an anisotropic crystal. In order to ascertain that the turbid melt is actually still a liquid, the cover glass is tapped with a pointed splinter. On cooling further, the transition from the crystalline liquid to the crystalline solid state takes place, which can also again be shown by testing with the splinter. Instead of *para*-azoxy anisol, a series of other related substances is usable. Attractive projection experiments.

Exercise 59. Aromatic Alcohols, Aldehydes, Ketones, and Acids

For the reasons stated under phenols, the following brief selection will suffice.

(*a*) As an example of phenyl hydrazone formation we recommend the action of benzaldehyde on *para-nitro phenyl hydrazine*: When the two substances are mixed on the slide a crystal mass appears after a short time; an excess of the aldehyde is to be avoided. The alcoholic solution of the nitrophenyl hydrazine also precipitates the hydrazone a short time after the addition of the benzaldehyde. A saturated solution of benzaldehyde which contains not even 0.3 per cent aldehyde shows a turbidity after a time and a precipitate of fine needles if the reagent is introduced into the solution.

(*b*) The oxidation of the side chain to carboxyl group can be easily shown with toluene: 0.5 mg. of toluene and 0.1 cc. Beckmann mixture (p. 121), which is diluted with three times as much sulfuric acid 1 : 5, are introduced into a

[1] If the stage has a large opening in the center, no further precautions are necessary as the congealing will proceed slowly enough. Otherwise, the slide is raised at both ends so that a sufficiently long portion in the center is free.

heavy-walled sealing flask (Fig. 72, p. 129). At the most, only one-fifth of the flask should be filled with the liquid. The oxidation takes place on heating to about 245°, i.e., in the amyl benzoate bath (p. 14) in which the flask remains for a minute; it is advisable not to place it directly in the vapor but rather in a brass protecting tube which is soldered shut at the bottom and immersed in the vapor bath. After cooling, which may be hastened by holding under the water tap, the protecting tube (with flask) is lifted out (goggles!) and cooled further under the tap. The flask is then opened and the contents brought under the microscope. Delicate leaves and needle clusters of benzoic acid, weakly polarizing.

(*c*) The saponification of esters and cyanides is carried out similarly. Capillaries (not too heavy walled) with maximum bore of 1 mm. may be used. They are observed immersed in water directly under the microscope.

(*d*) The *aromatic acids* present above all many beautiful reactions for microchemistry, owing to their remarkable crystallizing ability; many, e.g., may be *sublimed*. If the crystals so obtained are not well defined they can often be improved by breathing upon them or by recrystallization from hot water.

Instead of numerous details (see also Emich, "Lehrbuch d. Mikrochemie," Munich, 1926) it may be mentioned that cinnamic acid, for example, dissolved in carbon disulfide forms a splendidly crystallizing dibromide on treatment with bromine.

Exercise 60. Anthracene, Anthraquinone, Alizarin

Of the compounds with condensed nuclei we may use anthracene, anthraquinone and alizarin and select the following examples for practice.

(*a*) Conversion of anthracene to dianthracene. A saturated solution is prepared by grinding up anthracene with a drop of xylol. A thin-walled (e.g., melting-point) capillary is partly filled with the clear liquid and sealed at both ends. Then the tube is placed in front of an ultra-violet quartz lamp for 15 minutes or in direct sunlight (*outside* the window) for several days. Copious crystals of *dianthracene* appear. Observed in the cell (p. 15).

(*b*) Anthracene offers the possibility of carrying out a very pretty experiment with the *fluorescence microscope*. The sublimate obtained by the "slide to slide" procedure (p. 39) is used. Details in Emich; "Lehrbuch d. Mikrochemie," Munich, 1926, pp. 66, 67.

(*c*) The oxidation to anthraquinone is carried out by means of chromic acid in glacial acetic acid. Four milligrams of anthracene are heated with this mixture for 15 minutes in a sealed tube at 150°. The anthraquinone crystallizes in needles after being blown out on a slide and allowed to cool and stand in the air. The mother liquor is removed and the anthraquinone recrystallized from hot nitrobenzene. Observation in polarized light according to footnote 8, p. 8. Traces of anthraquinone are sought with crossed nicols because the smallest crystals also appear distinctly on account of their strong polarization.

(*d*) Conversion from anthraquinone *back* to anthracene by zinc dust distillation. A difficultly fusible glass tube (Fig. 73) of 0.5-cm. inner diameter is drawn out to a capillary such as is used in melting-point determinations; at *a*, a dis-

tance of 1.5 cm. from the capillary, it is constricted after a plug of ignited asbestos *b* has been introduced. Then a 1-cm. layer of pure zinc dust is filled in, and after that a 0.5-cm. layer of mixed zinc dust and substance. The tube is sealed at *a*, but the portion to the left remains attached, to be used for clamping the tube. First the zinc dust layer and then the mixture is heated with a small flame to a dull red heat. The product sublimes into the capillary. Finally the capillary is cut off and sealed at one end and the anthracene recrystallized therein, etc., as described above.

FIG. 73.—Zinc dust distillation.

(*e*) A fabric dyed with alizarin is extracted with a mixture of concentrated hydrochloric acid and alcohol, according to H. Behrens. The extract is evaporated and the residue sublimed (see below) or recrystallized from nitrobenzene. The currant-red, brightly glittering needles are dichroic (yellow-orange). Alkalies and ammonia dissolve them to give a purple solution. Aluminum salts precipitate a red lake from the solution. Acids precipitate brown flakes which can be sublimed at about 100°, according to the procedure in Exercise 61.

Exercise 61. Indigo

Indigo may be sublimed from fabrics, according to Rathgen or Kempf. If the apparatus described by the latter is not available, several threads dyed with indigo are laid on the copper (aluminum) block (Fig. 72, p. 129). By grinding the threads in the agate mortar with a drop of water and then pressing them on the block it is possible to make good contact between them and the block. After drying, a cover glass is placed over the sample so that it will form the upper wall of a very low sublimation chamber. The block is heated to 180 to 200° C. by a flame placed underneath.

Of course the object to be heated may also be placed on a slide. Duration of the experiment: 2 to 6 hours.

If indigo powder (or alizarin, etc.; see above) is to be sublimed directly, one proceeds in an analogous manner. After the powder, which has been moistened with water, has dried on the block, several glass threads bent, e.g., in the form of right angles, are laid on the latter, and finally the cover glass is placed over these.

The sublimed indigo crystals are (predominantly) distinctly pleochroic rods.

Exercise 62. Alkaloids

For the microchemistry of the alkaloids the reader is referred to the special literature.[1] As is known, the alkaloid reagents are divided into the general and specific.

[1] Above all, Molisch, Mikrochemie der Pflanze (1921), and Tunmann, Pflanzenmikrochemie (1913), where also further literature is cited. See also A. Mayrhofer,

(*a*) *General precipitating reagents* are tannin, potassium iodide-iodine, potassium mercuric iodide, potassium cadmium iodide, potassium bismuth iodide, phosphomolybdic acid, phosphotungstic acid and picric acid. In many cases the alkaloids may be recovered from the precipitates formed. For example, the iodine precipitate is dissolved in aqueous sulfurous acid after washing, and evaporated down to drive off the excess sulfurous acid and hydrogen iodide; the resulting solution (possibly after filtration) contains the alkaloid as sulfate.

For practice, a quinine sulfate solution may be used, a small drop of which is used in each test.

(*b*) Because of the enormous number of specific reactions, only a few can be selected (almost arbitrarily).

(α) *Cocaine.* A dilute aqueous solution of the hydrochloride is spread over the slide and a 1 per cent $KMnO_4$ solution introduced into the center of the drop. After about 5 minutes, finger- and hand-shaped crystals and spheres appear. Rosenthaler recommends as a pretty reaction the one with tetranitrito-diamino-cobalti-potassium.[2]

(β) *Atropine.* The alkaloid is heated with a drop of sodium hydroxide solution and the vapors are allowed to condense on a slide. Hydrochloric acid is added to the condensate, which is then allowed to dry up and is redissolved in a drop of water. On addition of potassium iodide, needles and rhombohedrons of *tropine hydriodide* 10 to 15 μ in size appear.

(γ) *Morphine.* A solution of the hydrochloride is acidified on the slide with acetic acid. Next to it is placed a drop of potassium iodide-iodine solution (e.g., 10 : 1 : 100); the solutions are joined by a thread of liquid and allowed to stand for 5 minutes. At first brown droplets appear, then pretty crystals which are reminiscent of silver dichromate.

(δ) *Quinine.* 1. The fluorescence experiment in the capillary is carried out according to p. 99, the solution being acidified with dilute sulfuric acid. 2. Preparation of herapathite: Water, alcohol and a trace of sulfuric acid are mixed and a long drop of the mixture placed on the slide. Some iodine is introduced in one end and some of the sample at the other. In order to prevent the drop from breaking, a glass thread may be laid in it. The slide is then covered with a watch glass and allowed to stand for 5 to 30 minutes. Rhombohedrons, prisms and aggregates of both are formed, showing very remarkable pleochroism (colorless or yellowish to violet-brown or black).

In the examination of drugs containing alkaloids (which does not come within the scope of this book), *vacuum sublimation* renders good service. The procedure is described in detail on p. 39. As object for the experiment, a little tea leaf is recommended (sublimation of caffein).

Mikrochemie d. Arzneimittel u. Gifte, Vienna and Berlin, 1923, I, 1928, II. Attention need not be called to the book of Behrens; for the beginner, Emich, Lehrbuch d. Mikrochemie, Munich, 1926, will suffice. In this book, remarks on the reactions of proteins are also given on p. 268.

[2] A. Mayrhofer, Mikrochemie d. Arzneimittel u. Gifte, Vienna and Berlin, 1928 II, p. 220. L. Rosenthaler, Pharmaz. Zentralhalle 67, 177 (1926).

Exercise 63. Molecular Weight Determination According to Barger [1]

Of the numerous methods for micro molecular weight determination, the procedures of Barger and of Rast will be described in the following. The former method, which is distinguished by its particular simplicity and which has proved satisfactory in determinations carried out in this laboratory, will be described in its original form.

Principle: If droplets of two solutions, one osmotically stronger than the other, are in a capillary, the concentration of the two tends to become equal in that solvent will pass from the more dilute to the more concentrated droplet. The stronger solution grows at the expense of the weaker, as can be seen under the microscope. With water it requires days, with alcohol hours, and with more volatile solvents minutes before the change can be noticed under the microscope. In this way a test solution can be compared with known solutions in an osmotic scale and the normality of the sample thus determined.

A great advantage of this method which is not found in others is that no painstakingly purified solvent is required; in fact, any mixtures at all can be used as solvents. It is only necessary that sample and standard solutions be prepared from the same liquid. Barger justly presents as the chief advantage of his method the possibility of using pyridine, in which almost all organic substances are readily soluble, which is very difficult to obtain sufficiently pure for ebullioscopic determinations and the freezing-point of which is inconveniently low for cryoscopic experiments.

Procedure: 1. *Solutions.* Various non-volatile substances may be used for the standard substance of known molecular weight. For organic solvents, azobenzene, benzil, β-naphthol, etc., are generally used. For the preparation of the scale of the standard solutions, a solution of known concentration is diluted with the help of a buret to 0.2, 0.4, 0.6, 0.8 molal, etc. Should these limits be too wide, intermediate concentrations such as 0.45. 0.5, 0.55, if the test solution is, e.g., stronger than 0.4 and weaker than 0.6 molal, may be prepared by corresponding dilution of the standard solution. The limit of error of the molecular weight determination is usually set by the error in the preparation of the solutions.

Test solutions of difficultly available substances can be prepared in small vials or tubes of 3- to 4-mm. bore and about 15-mm. length by weighing out the substance (several milligrams) and the solvent (50 to 100 mg.) in them. For use, the end is broken off and sealed again after taking out the required amount.

Long-necked ampules are suitable for preserving the standard solutions for further experiments. These have a capacity of about 2 cc. and a neck 16 cm. long and are wide enough to permit the introduction of a capillary. They are

[1] Ber. dtsch. chem. Ges. **37**, 1754 (1904); Abderhalden, Handbuch d. biochem. Arbeitsmethoden **8**, 1 (1915). For modifications of the Barger method see: K. Rast, Ber. dtsch. chem. Ges. **54**, 1979 (1921); A. Friedrich, Mikrochemie **6**, 97 (1928); K. Schwarz, Monatsh. Chem. **53/54**, 926 (1929); R. Signer, Liebigs Ann. **478**, 246 (1930); E. Berl and O. Hefter, Liebigs Ann. **478**, 235 (1930). The attention of the reader is called particularly to the last paper.

made from a glass tube of 1.5-mm. width by blowing up a ball at the end. To fill the ampules, they are evacuated and the neck closed by fusing at a previously constricted point. Pushed into a vessel, these ampules fill automatically when the tip is broken off. Pyridine solutions are suitable for very many purposes. Pyridine requires from 1 to 4 days' waiting. With such (not very volatile) solvents (also water, formic acid, etc.) the equalization may be hastened by heating.

2. *Capillaries.* The capillaries are made from a rather thick-walled glass tube about 15 mm. wide which is drawn out into capillaries of about 1- to 2-mm. bore. Pieces of these capillaries 10 to 15 cm. long are used.

3. *Filling and Measurement of the Droplets of Liquid.* The filling of the drops into the capillaries requires a certain amount of practice, which is soon acquired, however. The tube is held between the thumb and middle finger and while the upper end is closed with the index finger the lower end is dipped into the solution of standard substance of known molecular weight. The pressure of the index finger on the capillary is lessened, and thereby a little column 5 to 10 mm. in length is allowed to enter. Then the upper end is again closed with the finger; the capillary tube is removed from the liquid and inclined until the filling end is higher than the closed end; the pressure of the finger is lessened, so that the column slides down the capillary until it is about 2 to 3 mm. from the filling end. The tube is then again closed with the index finger and the liquid on the outside of the tube is wiped off; then the surface of the test drop containing the substance (the molecular weight of which is to be determined) is touched with the filling end. This time the pressure of the index finger is hardly lessened, so that no air leaves the tube and only a very small biconcave drop enters. The tube is again raised to an inclined position and the drop allowed to slide 2 to 3 mm. into the tube as before. Then in the same way a drop of the standard solution is taken up, etc. After 2 to 4 small drops of each solution, a column of 5 to 10 mm. of standard solution is again allowed to enter, and since the capillary action is usually not sufficient for this, the capillary is dipped deeper in the solution and the amount of entering liquid regulated by the finger. When all the drops have been introduced they are allowed to slide down the tube until the last column is about 1 cm. from the filling end, which is then sealed. The other end of the capillary is sealed 1 to 2 cm. from the drop first introduced. For more convenient manipulation, the tube is then fastened to a slide by means of wax or plasticine or fixed to it with rubber bands. In Fig. 74, the black drops are the standard solution; the others, the solution containing the substance the molecular weight of which is to be determined.

Only the small droplets are measured. The two larger columns (the first and the last introduced) serve for sealing and change irregularly at the fusing of the tube and because of evaporation into the air spaces at the ends.

For measurement, the slide together with the tubes is laid in a flat glass dish (which can be made by fastening four glass rods with wax to the edges of a glass plate about 4 cm. wide and 20 cm. long), and water is added until the tubes are just covered.

The choice of the droplet size is determined on the one hand by the desire for

the greatest possible accuracy in measurement and on the other hand by the fact that the length of the droplet must not exceed the length of the micrometer scale. When the capillary is filled in the manner described above, objective 3 of Leitz in conjunction with ocular 4 or Zeiss ocular 2 and objective A, for example, may be used (magnification of 60 to 70 times). The microscope is focused on the axis of the capillary; the two menisci of the droplet to be measured are then sharply defined. The scale of the micrometer should be parallel to the capillary. Fig. 75 shows the picture under the microscope.

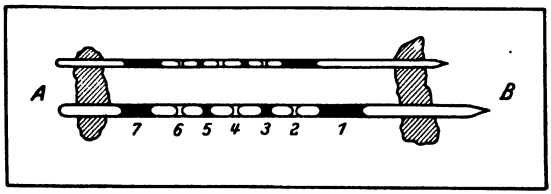


FIG. 74.—Barger's molecular weight determination.

4. *Experiments.* Azobenzene is recommended as standard substance; as test substance, naphthaline or urea; as solvent, acetic ether or alcohol. A 0.45 molal solution of the test substance is taken, a series of standard solutions of the azobenzene is prepared, i.e., 0.1, 0.2, 0.3, etc., to 0.7 molal solutions. The experiment is carried out by comparing in the first capillary the test solution with the 0.1 molal standard solution; in the second capillary, with the 0.2 molal solution, etc. It will be seen that in the fourth capillary the 0.4 molal solution still gives off solvent to the test solution whereas in the fifth capillary the 0.5 molal solution takes up solvent. For many purposes this determination will be sufficient. The tests may be continued with the series 0.42, 0.44, 0.46, 0.48.

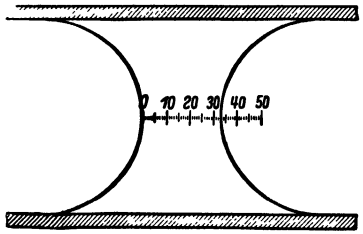


FIG. 75.—Microscopic image of drop and eyepiece micrometer scale.

Because of its great accuracy and—as Barger has already pointed out—independence of the particular properties of the solvent, the method described is suited to investigation of association.

Exercise 64. Molecular Weight Determination According to Rast

Rast found in *camphor* a solvent which was distinguished by such a high molecular depression that it offered the possibility of using an ordinary thermometer divided into whole degrees instead of a Beckmann thermometer, and of making the measurement in an ordinary *melting-point apparatus.* The freezing-point depression of camphor is 40° for 1 mole in a kilogram of solvent; the corresponding value for, e.g., benzene is 5° and for water only 1.86°. The camphor also possesses a remarkable solvent power. The melting-point is determined with the usual precautions, which are referred to on p. 32. The procedure

has been modified by A. Soltys [1] so that one can work with *fractions* of a milligram.

First procedure according to Rast: "Several milligrams of substance are fused with 10 to 20 times as much camphor in a very small test tube previously cleaned with dichromate and sulfuric acid; some is removed from the congealed melt cake by means of a microspatula[2] and its melting-point determined.

"The test tube is set on the balance in the hole of a cork. After the substance has been weighed in, the tube is sealed with a cork through which a pointed knitting needle is inserted. The contents are fused and mixed by immersing the tube in hot sulfuric acid or paraffine. This requires only a few seconds. The traces of camphor which sublime thereby were formerly weighed back after being removed, but it was shown that they never caused an appreciable error. The mass is then picked out (the characteristic softness of the camphor is of great advantage here) and placed in an agate mortar or watch glass. A thin-walled melting-point tube is pressed against the kernels, and these are pushed down into the tube by means of a glass rod and pressed together. The tube is introduced into the side opening of a melting-point apparatus, or better, drawn out 2 cm. above the substance into a capillary about 15 cm. long and fastened to the thermometer with sulfuric acid by means of this capillary.

"Long before the melting-point is reached the mixture begins to look like thawing ice. It finally becomes a turbid liquid in which, by the help of a magni-

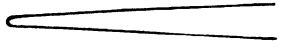


FIG. 76.

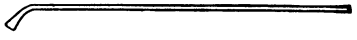


FIG. 77.

fier, one can see a delicate crystal skeleton which permeates the entire melt in the beginning but dissolves from the top downwards on slow temperature rise. The disappearance of the last crystals indicates the correct melting-point."

According to Rast, it is superfluous to apply corrections for emergent stem or use a normal thermometer, since only difference determinations are concerned.

On the other hand, J. Houben, who showed the applicability of the method to liquids which are not too volatile, recommends the use of a normal thermometer since the usual type often shows unequal intervals.

Several samples are worked with (acetanilide, M.W. = 135, picric acid, M.W. = 229, etc.); 2 to 3 mg. suffice for one determination.

Second Procedure. Preparation of the solutions: "It has been shown that the capillaries may be somewhat wider without endangering the accuracy, namely, with a bore of 2 to 3 mm. Besides this, they are widened conically toward the open end so that they have the form shown in Fig. 76. On the other hand, thin walls and rounded-off bottom are essential as before. A microspatula (Fig. 77) is used for filling.

[1] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia 1930, p. 219.

[2] Made easily by hammering flat and filing the end of a hard brass wire.

"The capillary is set vertically in a hole in a cork (outside of the microbalance). The substance must fall freely from the spatula to the bottom of the capillary. The camphor is then added, the kernels of which are pushed off the walls by means of a glass rod with unpolished edges, and pressed together on the bottom. This may be done very neatly. The capillary is weighed at intervals, laying it each time on the microbalance. Finally, the capillary is sealed and drawn out as usual to a thread which is fastened to the thermometer with sul-

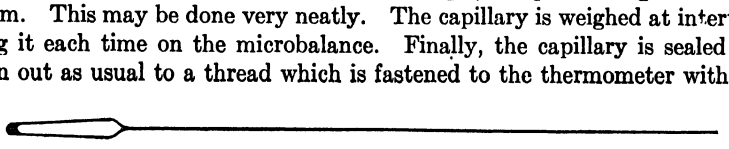


FIG. 78.

furic acid (Fig. 78). By melting and allowing to congeal again the contents are mixed.

"For holding the capillary while weighing, a Holtz forceps [3] is used or a short glass rod which is fused to the bottom and which may be touched with the finger tips (Fig. 79). The glass pieces illustrated here may be made without difficulty by drawing out a test tube over the wing-top burner and cutting with a sharp glass knife; they are, of course, kept painstakingly protected from dust. A filling funnel like Fig. 80 occasionally renders good service.

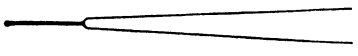


FIG. 79.

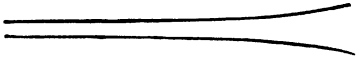


FIG. 80.

FIGS. 76-80.—Molecular weight determination according to K. Rast.

"A thickening of the bottom of the capillary is usually unavoidable and does no harm.

"The height of the melt in the capillary should not exceed 2 mm. Three millimeters, in fact, involve some danger of error. Therefore as little substance as can be introduced into the capillary ($\frac{1}{3}$ to $\frac{1}{5}$ mg.) is taken, and as a rule 2 to 3 mg. camphor."

For practice material, Rast recommends naphthalene, sulfonal, acetanilide. Molecular weights of, e.g., 126 (instead of 128) 225 (228), 142 (135) are obtained.

Quantitative Exercises

Exercise 65. Testing of Balance and Weights

The procedure described on p. 54 is followed. The influence of the surroundings is especially to be determined, e.g., the zero point is repeatedly determined while the heating plant is in operation, when the sun shines into the room, people pass by, machines run in the building and

[3] Obtainable from Bender and Hobein, Munich.

trucks pass in the street. These influences do not disturb as a rule; still, when they are present, they vary in different institutes, and therefore they must be determined for each particular case.

The 1-cg. weight and the two 2-cg. weights are standardized against the rider (which is assumed to be correct). The latter is therefore placed in the notch "0" and the zero point brought to "0" on the pointer scale. The 1-cg. piece is then placed on the left pan of the balance and the rider on the mark, "10" and the relation between the two is averaged by repeated readings. One proceeds in an analogous manner with the other weights mentioned above. The results are collected in a table if necessary.

Residue Determinations

Exercise 66. Barium Chloride

(*a*) Preparation of a Donau dish according to p. 75. Of course the perforation of the foil and preparation of a filter bottom are omitted. But it is well to prepare a cover at the same time from a small piece of foil.

(*b*) *Residue Determination.* From 5 to 10 mg. of the powdered salt (from crystals selected under the microscope) are weighed out in the covered dish and heated to dull red heat on a suitable base (p. 65). The dish is allowed to cool off for 2 minutes on a metal base (small block of copper or aluminum) in the desiccator, then allowed to stand for 1 minute beside the balance pan and finally weighed. Content of water: 14.7 per cent.[1] For such determinations the Nernst balance is very convenient. See p. 61.

In place of the dish of foil a small crucible with cover may also be used; naturally the necessarily longer time of cooling must be taken into consideration.

Exercise 67. Determination of Potassium as Sulfate

Five to ten milligrams of pure *potassium acid tartrate* are weighed out in the micro platinum crucible, and the sample is converted to the sulfate according to p. 66. The treatment of the potassium sulfate with ammonium carbonate (or fuming with ammonia) is to be repeated until constant weight is obtained. In order to prevent at least partly the creeping of the sulfate, a piece of platinum gauze or the like (of course weighed with the empty crucible) may be placed in the crucible. For the reason given, the Donau dish is not so practicable. The experiment may be carried out in the Pregl micromuffle, Fig. 51, p. 67. Potassium content: 20.8 per cent.

[1] Concerning the varying water content, see Hüttig and Slonin, Z. anorg. Chem. **181**, 69 (1929).

Exercise 68. Platinum Determination in a Chloroplatinate

It is first ascertained, by a qualitative experiment, that the decomposition by heating does not proceed in a violent manner. In this respect the quinoline compound is a convenient preparation, the ethyl amine compound an inconvenient one. But good results may be obtained also with the latter if it is placed in a covered Donau dish and this heated very gradually in a porcelain crucible. Platinum content of the ethyl amine compound: 39.0 per cent. With dearth of material it is advisable to prepare the chloroplatinate in a small porcelain crucible and filter by means of the filterstick p. 68. Then the preparation need not be transferred to another vessel.

Precipitation Analyses

The following collection includes practice exercises which were carried out frequently in the course given by Dr. Benedetti-Pichler and which were collected essentially by him and Dr. H. Alber. Sample, if not specified otherwise, means 5 to 10 mg. The determinations are carried out:

(*a*) in glass beakers with asbestos-mat filtersticks, Exercises 69, 70, 71, 72 (calcium), 73 (silver);

(*b*) in porcelain crucibles with quartz filtersticks, with asbestos mat, Exercise 72 (magnesium);

(*c*) in porcelain crucibles with porcelain filtersticks, Exercise 73 (copper).

Exercise 69. Determination of Aluminum as Oxy-quinoline Compound [1]

One to six milligrams of potassium alum (corresponding to *0.1 to 0.6 mg. aluminum oxide*) are weighed out in the microbeaker, dissolved in about 1 cc. water and a drop of concentrated hydrochloric acid and 0.3 cc. "oxine reagent" added. It is then placed on the boiling water bath and 2 *N* ammonium acetate added, dropwise, to the first permanent turbidity, and after a minute, during which the turbidity tends to become crystalline, 0.5 cc. more of the acetate solution is added, dropwise. All volumes given are sufficient for 1 mg. aluminum oxide; if more aluminum is present the amounts of solutions must be enlarged accordingly. After conclusion of the precipitation the beaker is allowed to stand 10 minutes longer on the boiling water bath. Then the solution over the precipitate is drawn off hot through the filterstick and the precipitate sucked as dry as possible. It is washed four to five times with 0.25 to 0.5 cc. hot water. The asbestos-mat filterstick is particularly suitable for the handling of the precipitate. The filtration and

[1] Benedetti-Pichler, Mikrochemie, Pregl-Festschrift, p. 9 (1929).

washing are easily completed in 5 minutes. The precipitate is then heated in a stream of air at 140° in the drying apparatus described on p. 72. After this the beaker and filterstick can be prepared for weighing in the usual way. *The time of drying is shortened at least 3 hours by working with small quantities.*

The "oxine reagent" is prepared by grinding 5 g. 8-oxy-quinoline with 12 g. glacial acetic acid, adding 83 g. of water and warming gently if necessary.

Theory: 10.77 per cent Al_2O_3.
Absolute error usually less than 0.05 per cent Al_2O_3.

Exercise 70. Determination of Nickel as Glyoxime Compound

Nickel ammonium sulfate is dissolved in about 2 to 3 cc. of water with the addition of a drop of concentrated hydrochloric acid, and, depending on the amount of nickel, 0.25 to 0.50 cc. of 1 per cent alcoholic dimethyl glyoxime solution is added so that an excess is certain. Then it is warmed carefully (because of the alcohol!) on the water bath and precipitated by the dropwise addition of dilute ammonia. The solution should become only weakly ammoniacal; since the test by odor in the presence of alcohol presents difficulties to some analysts, it is better to add some alcoholic methyl red. At the conclusion of the precipitation, it is allowed to cool for 15 minutes, then filtered and washed four to five times with hot water and possibly once with 40 per cent alcohol in order to prevent creeping of the precipitate. Finally it is dried for 10 minutes at 110° in a current of air. The determination may be carried out in a Schwarz-Bergkampf filter beaker[1] which has a filter disk of fritted glass.

Theory: 14.88 per cent Ni in $Ni(NH_4SO_4)_2 \cdot 6H_2O$.
Absolute error usually less than 0.1 per cent Ni.

Exercise 71. Potassium Determination as K_2PtCl_6 [1]

(*a*) Sample: from 1 to 3 mg. KCl (Merck pro analyse, recrystallized twice).

The substance is dissolved in 0.3 to 0.4 cc. water in the beaker and treated with H_2PtCl_6[2] in slight excess. It is evaporated to dryness on

[1] Z. anal. Chem. **69**, 336 (1926).

[1] We mention a few examples out of the large number of determinations in order to meet the objections of Lundegårdh to this method. See footnote 4, p. 53. The experiments were carried out by Dr. H. Alber.

[2] Prepared according to Treadwell-Hall, Analytical Chemistry, Vol. 2, 5th ed., John Wiley & Sons, New York, 1919–21.

the water bath, not too rapidly, by blowing on air, dissolved in 0.25 to 0.50 cc. absolute alcohol and filtered through the asbestos filterstick. The precipitate is washed two to three times with absolute alcohol and heated for 5 minutes in the drying block in a current of air at 150 to 160°. The potassium is calculated with the familiar empyrical factor 0.1603.[3]

Theory: 52.5 per cent K; found: 52.7, 52.3, 52.5 and 52.6 per cent K.

(*b*) In the presence of sodium[4] the sum of the chlorides is determined by evaporating two or three times with (residue-free) HCl and drying at 400°; the precipitation of the potassium is carried out as above, with, of course, a corresponding excess of H_2PtCl_6. After the addition of the absolute alcohol, the crystal mass is well crushed with the filterstick and washed until the filtrate is colorless. The potassium values are usually too high (about 0.6 per cent relative).

Sample: 2.206 mg. KCl, with about 2 mg. NacCl; found in KCl: 52.7 per cent K.

Exercise 72. Separation of Calcium and Magnesium

Calcium carbonate and magnesium oxide (or a solution of magnesium chloride) are weighed out and covered with 1 cc. water in a microbeaker. Five drops of concentrated hydrochloric acid are added; losses due to evolution of gas are avoided by holding the beaker in an *inclined* position. It is then heated carefully over the microflame of the Bunsen burner until the dissolved carbon dioxide (and possibly traces of chlorine from the hydrochloric acid) is driven out. After the addition of 0.5 cc. 3 per cent oxalic acid and a drop of alcoholic methyl red, dilute ammonia is added dropwise until the first turbidity appears; it is redissolved by heating and if necessary adding hydrochloric acid. Then the calcium is precipitated at boiling temperature with 2 per cent ammonia, which is added dropwise with constant agitation until the indicator changes. After standing for 1 hour (under a bell jar) it is filtered through the asbestos filterstick, washed four to five times with 0.5 cc. cold water, dried 10 minutes in a current of air at 105° (maximum temperature) and weighed as $CaC_2O_4 \cdot H_2O$.

The filtrate from the calcium precipitation[1] is caught in a porcelain crucible of 12- to 14-cc. capacity, which has been weighed together with the quartz asbestos filterstick. It is evaporated if necessary to 2 to 3 cc.,

[3] Küster-Thiel, Tabellen, p. 49 (1929).

[4] See also Treadwell-Hall, Analytical Chemistry, Vol. 2, 5th ed., John Wiley & Sons, New York, 1919–21.

[1] For the magnesium determination alone, $MgSO_4 \cdot 7H_2O$ is used as sample. It is best recrystallized freshly every time.

treated with 3 drops of concentrated hydrochloric acid and 1 cc. 5 per cent sodium phosphate solution and heated on the asbestos wire gauze to boiling. Then 10 per cent ammonia is added, dropwise at first until the indicator changes to yellow, then rapidly until sufficient has been added to give an approximately 3 per cent ammoniacal solution. After standing 12 hours under a glass bell jar it is filtered by means of the filterstick and washed five times with 0.5 cc. 3 per cent ammonia. The crucible is wiped off on the outside with a non-linty cloth, dried at 120° in the drying oven and finally ignited in the electric crucible furnace, in which the temperature is raised to about 1000° within 20 minutes. A slight gray color of the pyrophosphate does not noticeably affect the result.

On a perhaps necessary double precipitation of the oxalate and several other details see the original.[2]

Exercise 73. Separation of Silver and Copper [1]

Silver acetate and copper sulfate are weighed out in a microbeaker which has been weighed with the asbestos filterstick. They are dissolved on the water bath in about 1 cc. water and a drop of dilute nitric acid (1 : 4). The silver is precipitated with 5 per cent sodium chloride solution which is added until the last drop does not produce any precipitate. It is allowed to stand on the water bath for 15 minutes, cooled under the tap and filtered after standing again for 15 minutes. Washing four times with cold water or 1 per cent nitric acid will suffice; the silver chloride is dried at 120° for 5 minutes in a current of air in the Benedetti-Pichler apparatus (p. 72).

The filtrate is caught in a porcelain crucible of 12- to 14-cc. capacity which has been weighed with the porcelain filterstick. It is diluted if necessary with water until the crucible is about two-thirds full of liquid. After this it is heated to boiling by placing the crucible on an asbestos wire gauze and heating with the microflame of the Bunsen burner. The precipitation is carried out at the boiling temperature with 1 per cent potassium hydroxide solution which is added dropwise until the precipitate becomes dark and coagulates. It is then heated 3 minutes longer, allowed to settle and the supernatant liquid drawn off through the porcelain filterstick. It is washed four times with hot water, always allowing the precipitate to settle and decanting the supernatant liquid through the filterstick. Only with the last washing is the precipitate itself brought on the filterstick.

[2] Benedetti-Pichler, Z. anal. Chem. **64**, 420 (1924).
[1] Benedetti-Pichler, Z. anal. Chem. **64**, 418 (1924).

(If the copper hydroxide reaches the filter before the final washing, the filter may easily become clogged and the washing must be discontinued prematurely. This does not do any harm; the crucible and filterstick are dried, and ignited, and after cooling the precipitate may be washed with hot water without any difficulty.) The beaker is cleaned by wiping the outside with a cloth, drying at 120° for 5 minutes and igniting in an electrical crucible furnace for 10 minutes at 900°.

Exercise 74. Measurement of Small Amounts of Magnesium According to F. L. Hahn [1]

1. *Principle.* The red-violet alkaline solution of the 1, 2, 5, 8, tetrahydroxy anthraquinone (quinalizarin) changes color to cornflower blue on addition of magnesium ion. It is possible to identify 0.5 to 1 γ in 1 cc. with certainty. The tint of the magnesium solution is a combination of the red-violet of the alkaline dye solution and the blue of the magnesium lake. The lake forms flakes in higher concentrations; the magnesium-free sodium hydroxide solution cannot be kept unchanged. The solution to be measured ("sample") and the standard solution of known magnesium content must therefore be treated at the same time with dye and sodium hydroxide, and the sample checked against the series of standard solutions.

The solutions to be tested are compared in test tubes against a uniformly illuminated background which is best painted with aluminum bronze; a colorimeter block as used, e.g., for *p*H determinations according to Michaelis may also be employed. The insertion of a yellow filter may be advisable.

For concentrations of 0.5 to 10 γ per cc., 1.5 cc. test solution, 0.5 cc. 2 *N* NaOH and 0.2 cc. dye solution are used. The latter is prepared by grinding 0.1 g. dye intimately with 5 g. crystallized sodium acetate and dissolving 0.1 g. of this mixture in 20 cc. 96 per cent alcohol. This solution may be kept for a long time.

The range of greatest color change lies between 5 and 12 γ magnesium in the entire sample (3 to 8 γ per cc.); in this range steps of 1 γ are easily distinguishable. But the test is essentially facilitated if the standard solutions differ by double the amount, that is, 2 γ, and the concentration of the sample set equal to one of the standard solutions or expressed as lying just between two standard solutions. This decision can almost always be made with certainty at first glance. If one is not certain, one should look away from the test in order to permit the eyes to rest, and then make another observation.

2. F. L. Hahn recommends the following *method of working:* 0.6, 0.8 and 1 cc. of an original standard solution containing 10 γ magnesium in 1 cc. are measured off and the volume brought in each case to 1.5 cc. with distilled water. Then 0.1, 0.2, 0.4, 0.8 and 1.5 cc. of the sample, if entirely unknown, are measured off and all these samples are brought to 1.5 cc. with distilled water. First dye and then sodium hydroxide solution are added to the

[1] Mikrochemie, Pregl-Festschrift, 127; see also Ber. dtsch. chem. Ges. **57**, 1394 (1924).

tubes and they are then compared. In this way a first approximation may be obtained for contents between 4 and 100 γ magnesium per cubic centimeter. Then several samples are measured off which are divided fairly equally over the range 6 to 10 γ magnesium according to this approximation. These are compared with new standard solutions. The procedure is repeated if greater accuracy is demanded and the average of all the values taken. Using this procedure, Hahn finds, e.g., 5.7, 5.9, 6.0 γ instead of 5.8 γ.

If magnesium is to be determined in alloys with aluminum, the alloy must not contain more than 2.5 parts of aluminum to 1 part of magnesium. With unfavorable proportions, *hydrogen chloride gas* is led into the aqueous, ice-cooled solution whereby $AlCl_3 \cdot 6H_2O$ is precipitated and the liquid drawn off through a dry, fritted-glass suction filter. The required amounts are taken from the filtrate by means of a pipet and brought to dryness on the water bath, then dissolved to 100 cc. and worked with as described above. One can also work with ether and hydrogen chloride gas instead of hydrogen chloride gas alone, whereby, e.g., 20 cc. of the first solution are treated with 20 cc. ether in a 50-cc. volumetric flask, cooled with ice. The gas is led into the water layer in a lively stream by means of a narrow tube. After saturation, the flask is filled with ether and the solution drawn off with weak suction. Two portions are taken with the pipet from the filtrate; these are evaporated and the procedure followed as before with the residue.

Hahn has also worked out a procedure for extremely small amounts of magnesium (to about 0.001 γ) for which the reader is referred to the original.

Exercise 75. Electrolytic Determination of Copper

Of the numerous electrolytic micromethods[1] the procedures for the determination of copper have found the greatest approval. The apparatus proposed by Pregl[2] will be described first. It must first be mentioned that Pregl does not stir the liquid mechanically, but keeps it boiling gently during the electrolysis. The current is broken only after the liquid has cooled.

"The most important of the requirements[3] for carrying out the process are the two electrodes. For cathode a cylindrical gauze electrode of platinum (Fig. 81*K*), 10 mm. in diameter and 30 mm. long is used. As shown in the illustration this is welded longitudinally to a heavy platinum wire which projects 100 mm. over the upper end (of the electrode). In order to prevent the electrode from

[1] The literature is cited for the most part in Emich, Lehrbuch d. Mikrochemie, Munich, 1926, p. 107. From here it can be seen that the first work on microelectrolysis was published in the year 1904 by Jänecke, which does not appear to be generally known.

[2] Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 167.

[3] Obtainable from A. Orthofer, University of Graz; Wagner and Munz, Karlstrasse 42, Munich; or American Platinum Works, Newark, N. J.

coming in contact with the walls of the cell during its withdrawal, three glass beads 1.5 mm. in diameter are fused to both the upper and lower edges. It should be noted that so-called lead glass is not suitable for this purpose, since even with these small amounts, this material will be appreciably dissolved by the boiling during the electrolysis and thereby cause a negative error. A platinum wire (Fig. 81*A*) 130 mm. long is used as anode. It is bent as shown in the drawing, and two Y-shaped glass extensions are fused onto it, which serve to keep the anode in a definite central position within the cathode and to prevent it from coming in contact with the latter on withdrawal. . . . The electrolytic cell consists of a simple test tube, 16 mm. in outer diameter and 105 mm. in length, which may be clamped in the arrangement shown in Fig. 82. Here the cell may be conveniently adjusted, vertically and laterally, and the bent ends of the electrodes dipped into the mercury contacts, which are in turn connected with the source of current.

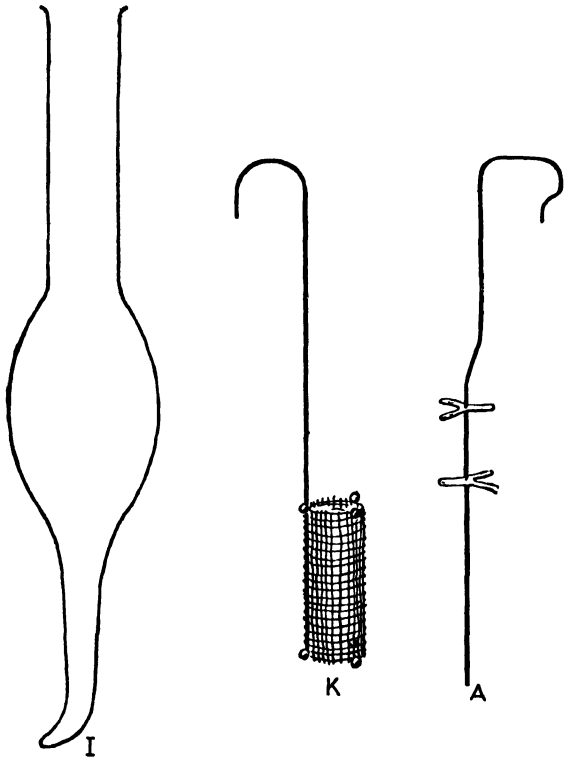


FIG. 81.—Electrolytic determination of copper. (Natural size.)

I. Internal condenser.
K. Gauze cathode.
A. Anode.

"It was found during the first experiments that small losses occurred through spattering of the liquid or through drops adhering to the wall of the empty part of the cell. This fault can easily be overcome by placing a loosely fitting internal condenser (Fig. 81*I*), with a laterally deflected tip in the mouth of the cell. This is made from an ordinary test tube by blowing a bulb in the middle and drawing out the closed end to a tip about 50 mm. long, as shown in the drawing. After any grease from its outer surface has been carefully removed with chromsulfuric acid, it is filled with water and used as shown in the drawing.

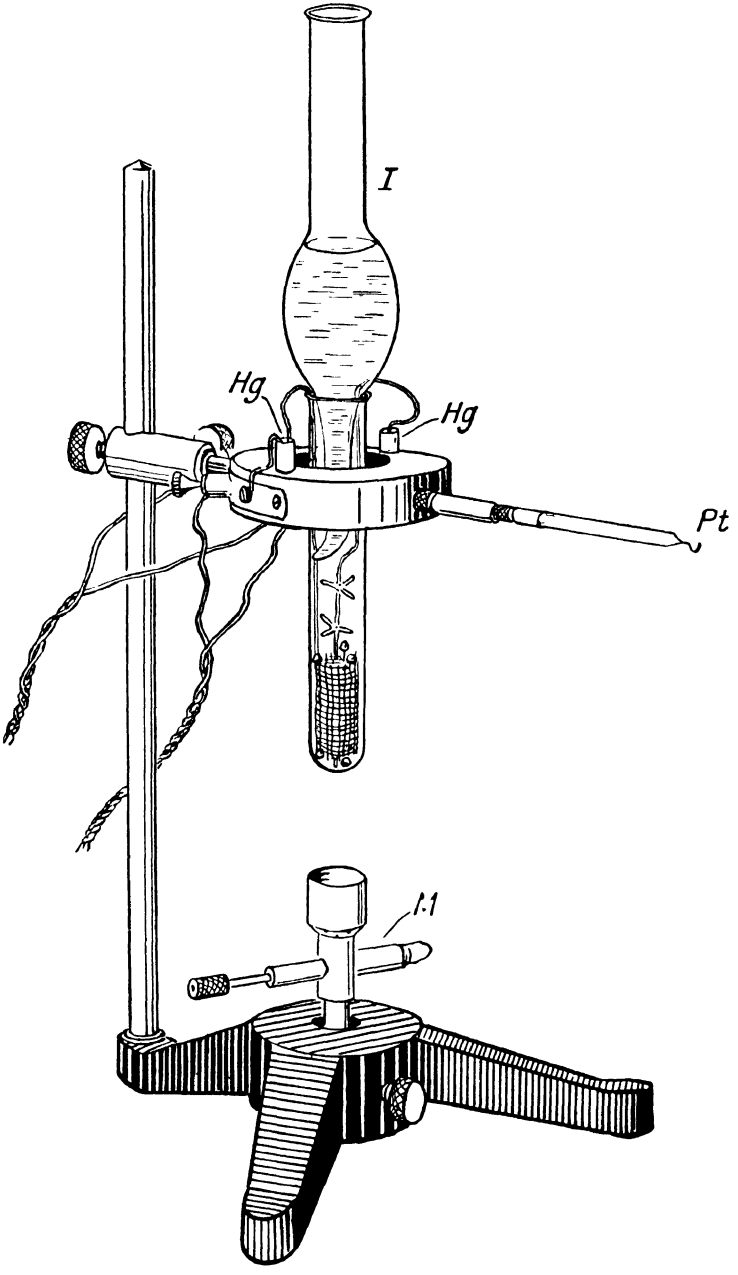


FIG. 82.—Apparatus for electrolytic determination of copper.

(½ natural size). *I*. Internal condenser. *Hg*. Mercury contacts. *Pt*. Platinum hook. *M*. Microburner.

"As source of current it is best to use two storage batteries. In the circuit are inserted (1) a resistance, (2) a reversing switch, (3) a voltmeter as shown in the accompanying wiring diagram (Fig. 83).

"The electrolytic determination of copper is begun by first dipping the platinum cathode, whether covered with copper or not, successively into concentrated nitric acid, water, alcohol and finally pure ether, and then drying it high above a Bunsen-burner flame. The electrode can be weighed after a short interval, because of the low heat capacity and high conductivity of platinum. For cooling it is hung on a platinum hook which is sealed into a glass rod attached to the microelectrolysis apparatus (Fig. 82*Pt*). The cathode is of convenient shape for standing on the left pan of the balance, on which it is supported by the three lower glass beads. The cell and condenser are cleaned with chromsulfuric acid and rinsed with water. When introducing the liquid to be electrolyzed into the cell, one should be careful that it does not rise to a height of more than 35 to 40 mm. from the bottom. The weighed cathode is next introduced, and then the anode, and their free ends are dipped into the proper mercury contacts. Finally the cell is closed by the condenser full of cold water, care being taken that the lower tip of the latter touches the wall of the cell so that the condensed liquid will flow back continuously. After the circuit is closed, the voltage is adjusted to 2 volts by regulation of the resistance and the cell is heated from below with the small microflame. The oxygen evolved at the central anode prevents any superheating, so that the liquid boils in a lively manner without bumping. It is advisable to slip the cell through a hole in a sheet of mica up to the meniscus of the liquid so as to prevent heating of the upper portions.

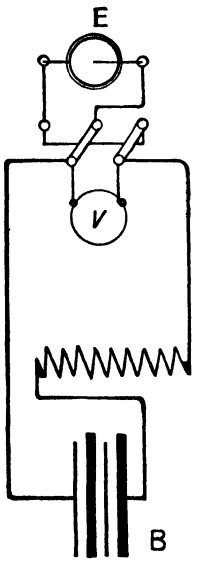


FIG. 83.—Wiring diagram for electrolytic copper determination.

E. Electrolysis cell.
V. Voltmeter.
B. Battery.

"Should the voltage change during the experiment, it is brought back to 2 volts by adjusting the resistance. In 10 to 20 minutes one may safely assume that the last traces of copper have been deposited on the electrode. This may be confirmed by testing with potassium ferrocyanide.

"The analysis is concluded by immersing the cell, through which the current is still passing, in a beaker of cold water and then, after a few minutes, in a second beaker. The apparatus is very convenient for this purpose, since by adjusting a single clamp the whole cell can be transferred from the flame to the cooling water without interrupting the electrolysis. When the contents are thoroughly cooled, the condenser is removed. Then, after carefully washing the hands, the anode is grasped with one hand and the cathode with the other; first the anode and then the cathode are withdrawn, avoiding any lateral movement while removing them from the cell. The cathode with its copper deposit is dipped first in distilled water, then in

alcohol and finally in ether, dried high over the Bunsen flame and hung on the platinum hook. After cooling it is again weighed.

"In the determination of copper in preserved foods, the first copper deposit on the electrode obtained as described above is usually contaminated with other metals, especially iron and zinc, but also with adhering traces of silicic acid. For this reason the weighed electrode is replaced in the cell, which has meantime been rinsed and filled with 5 cc. of water to which a drop of dilute sulfuric acid has been added. By reversing the current the copper is completely redissolved until the gauze electrode resumes its original color, and then the copper is redeposited on the cathode in the described manner. The copper deposit which is now obtained is no longer dull and discolored but has the typical color and luster of pure copper. In such cases the weight of copper obtained by the second electrolysis is always smaller than that of the first deposit, and by repeating the process once more it can be shown that the second value is reproducible to within 0.005 mg."

Benedetti-Pichler has carried out the electrolysis in dilute nitric acid solution instead of sulfuric acid solution, whereby it is possible to use the procedure for the analysis of various alloys. The Pregl apparatus has proved adaptable to the deposition of other metals, e.g., gold, silver and mercury.[4]

Clark and Hermance [5] have recently described a microelectrolytic apparatus which has several advantages over the Pregl set-up. The washing of the electrodes is considerably more reliable, among other things, and the electrolyte may be quantitatively and easily transferred after the deposition to another vessel for further analysis.

"The cell (Fig. 84) contains 4 to 5 cc. of electrolyte. The large outer vessel which is sealed to the electrode compartment at its base is a water-jacket for controlling the temperature of the electrolyte. Heating is effected by applying a microflame to the projecting arm and a thermometer is inserted as shown. Fig. 85 I, shows the glass frame which in the assembled cell fits inside the electrode vessel and serves to support both the electrodes and the pumping device. Its lower end is flared to form a conical bell having a diameter about 4 mm. less than that of the container. It will be observed that this frame is so constructed that, while the two electrodes are very close together, the possibility of their making contact or of becoming fouled by touching the glass wall of the container is eliminated. The glass beads fused around the base of the frame also insure a free space for the circulation of the electrolyte. This circulation is accomplished by the device illustrated in Fig. 85 II. This tube is provided with two flattened bulbs, a lower which fits loosely into the cylinder and operates as a baffle to arrest any spray, and an upper, also loosely fitting, which helps maintain the vertical position of the unit. Below the lower baffle, the tube is drawn into a heavy-walled capillary of about 0.5-mm. bore and 1.5-mm. outside diameter, which terminates below the bell on the inner unit.

[4] See Mikrochemie **1**, 86 (1923); **2**, 157 (1924); Pregl-Festschrift 46 (1926); Pregl-Fyleman, Quantitative Organic Microanalysis, Blakiston, Philadelphia, 1930, p. 173.

[5] J. Am. Chem. Soc. **54**, 877 (1932). Inserted by Translator.

"To operate this pump a stream of bubbles of air or of inert gas, introduced by means of the tube in Fig. 85 II into the space under the bell, causes a continuous overflow of liquid over the top of the tube *A* that supports the inner electrode. At the same time the suction caused by the removal of the liquid from under the bell further assists circulation in this direction by drawing upon that portion of the electrolyte in the space between the outer electrode and the wall of the containing vessel. A rapid agitation is thus maintained whereby the whole of the electrolyte is forced through the space between the electrodes several times a minute.

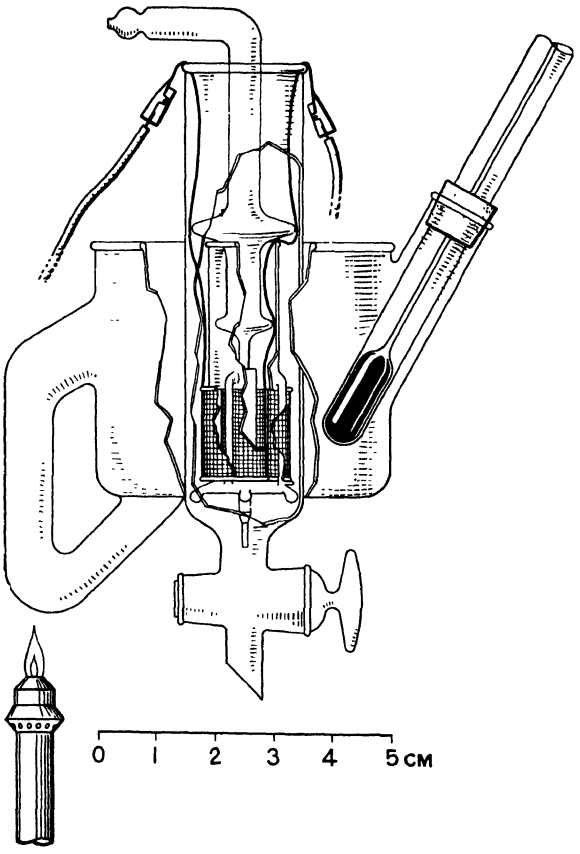


FIG. 84.

"The electrodes are two platinum gauze cylinders 15 mm. high, the one having an inside diameter of 9 mm., the other, 18 mm., weighing 0.8 g. and 1.5 g. respectively. . . .

"The electrodes are first cleaned by immersion in hot, concentrated nitric acid, followed by rinsing with distilled water. They are next heated to dull redness in the oxidizing portion of a small Bunsen flame, then cooled and weighed. The electrodes should, of course, always be handled with suitable forceps.

"After the electrodes have been weighed, the smaller cylinder is slipped over the inner, central tube (*A* in Fig. 85 I), the larger one over the three vertical supporting rods which form the cage. Thus assembled, the inner unit is lowered into the cell vessel containing the solution to be electrolyzed, the volume of which is then so adjusted that its level coincides with the upper end of the tube *A* in Fig. 85 I. The air tube is now introduced as indicated and a stream of bubbles admitted at such a rate that the liquid may be seen to flow regularly over the top of the tube *A*. Too much air

should, of course, be avoided, since it may cause excessive spraying of the electrolyte. Finally the platinum lead wires of the electrodes are connected, by small spring clips, to the flexible conductors leading to the current supply. The apparatus is now ready for the electrolysis. . . .

"The electrodes are washed by opening the stopcock at the bottom, while admitting distilled water from the top at the same time, the circuit remaining closed during the operation. When the complete displacement of the electrolyte is indicated by the fall of the current to a negligible value, the electrodes may be safely removed.

"If the excessive dilution of the electrolyzed solution involved in this method is objectionable, the following procedure may be used. The device shown in Fig. 85 III, which minimizes the quantity of wash water when only the inner electrode is to be weighed, consists of a glass tube, of outside diameter slightly less than the diameter of the electrode. The lower end terminates in a short, solid projection which fits loosely into the central overflow tube *A*. For a distance of 1 cm. from the end, the walls of the tube are perforated by a number of small holes. The open end of this tube is connected by rubber tubing to a reservoir containing distilled water, the flow being controlled by a suitable pinchcock. At the completion of the electrolysis, the air tube is withdrawn from the cell and this special washing tube inserted. The cell is drained slowly, while the wash water is admitted at the same rate through the perforations. At the same time, and without interrupting the current, the electrode is drawn up over the tube *A* until it occupies a position level with the perforations on the washing tube. The electrode, held in this position, is withdrawn together with the washing tube from the electrolyte; at the time it is pulled through the liquid surface it is surrounded by nearly pure wash water. If the holes in the washing tube are sufficiently fine and these indicated operations are executed rapidly and skillfully, the electrolyte suffers a minimum amount of dilution.

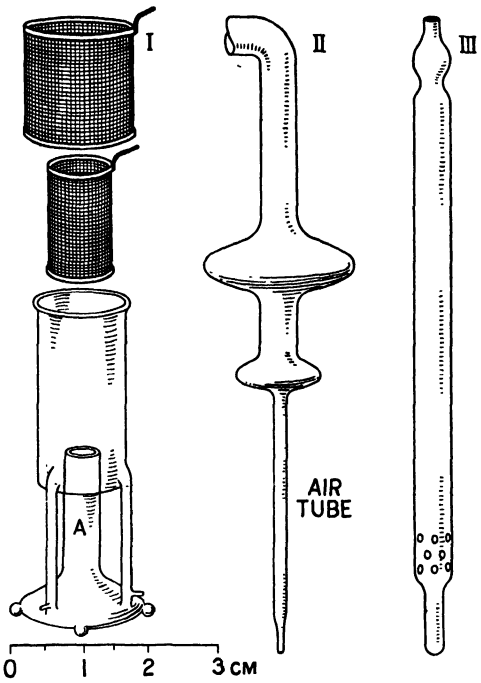


FIG. 85.

"After removal from the cell, the deposits are further washed with redis-

tilled alcohol and ether and dried for 2 to 5 minutes in an oven at 90° C. They are then protected in stoppered test tubes until weighed."

Clark and Hermance used this apparatus for the determination of Ni, Sn, Zn, and Cu. The determination of tin will be given as an example.

"As recommended by A. Fischer,[6] the electrode is first prepared by depositing a thin coat of copper, and then one of tin from an oxalate solution. The electrolyte consists of ammonium monosulfide solution containing sodium sulfite (to prevent formation of free sulfur). The best conditions for the electrolysis are: E.M.F., 1.2 volts (1200 milliamperes); time, 20 to 30 minutes; temperature, 60° C. After the electrolysis the electrode is washed with water, alcohol, carbon disulfide, alcohol and ether, in the order given."

The same authors describe in this paper an apparatus for the determination of small amounts of metals in large volumes of solutions by an electrolytic method. For details of the apparatus and further details of the described set-up, the reader is referred to the original.

Exercise 76. Volumetric Analysis

The volumetric solutions mentioned on p. 76 are prepared and standardized with 5 to 10 mg. of pure sodium carbonate.

[6] Z. anorg. Chem. **42**, 382 (1904).

SPOT ANALYSIS

By Dr. Fritz Feigl

(Privatdozent at the University of Vienna)

1. General

By spot analysis is meant a technic in analytical chemistry which permits the identification of certain substances by the combination of a drop of the test solution or minute quantity of solid substance with one or more drops of a reagent solution. In principle, almost all transformations of value in analytical chemistry can be carried out with adequate concentration of the reactants in the form of spot or drop reactions instead of in a test tube and the change observed in this way. In *practice*, however, only those chemical transformations come into consideration which, because of their sensitivity, take place when small amounts of the reactants are used, and also permit the detection of a certain substance in the presence of large quantities of other substances. Spot detections which are based on sensitive and specific reactions can then be used not only as identification reactions, for example in the course of the ordinary analysis, but occasionally for the solution of special problems (purity test, etc.). It is, however, also possible to employ reactions which are not decidedly specific if disturbing constituents are previously removed. The directions for separations (precipitation, filtration, and purification of small quantities of precipitate) can also be followed when only small quantities of sample are used as shown in the foregoing chapters.

Spot detections are of microchemical importance if, despite the use of macro drops of a test solution, the quantities of substance which may be detected by them are as small as by the methods of crystal precipitation, thread coloration, etc. Such microchemically valuable spot detections are therefore based throughout on reactions of high sensitivity and low limit of identification.

Spot reactions may be carried out on non-porous bases such as porcelain spot plates, watch glasses and micro porcelain crucibles, as well as on porous bases such as fabrics or filter paper. By carrying out spot reactions on paper, decided advantages are sometimes obtained by the utilization of the capillary properties of the paper. The different rates

of diffusion of water and the substances dissolved in it in the capillaries of the paper often bring about a concentration of a dissolved constituent in definite zones on placing a drop of an aqueous solution on filter paper. This may be of considerable value in the detection of small amounts of substances. Disregarding the thickness of the paper, such reactions may be said to take place in the plane of the paper. The original white color of the paper thereby essentially facilitates the observation of small amounts of precipitate or colored reaction products. If precipitation reactions are carried out on paper, a precipitation in the paper takes place first, followed by a further capillary diffusion of the constituents not precipitated. A sort of filtration is thereby carried out in the plane of the paper, and spot tests can easily be made in the border zones arising in this way.

In numerous cases the application of *papers impregnated* with suitable reagents presents distinct advantages. If perhaps a drop of salt solution which gives an insoluble precipitate with the reagent is placed on such a paper, the reacting constituent is held back in the center of the spot while the other ions diffuse further and may be detected in concentric circular zones. If a very dilute solution is used, a precipitate is not, as a rule, formed in the center of the spot, but instead the very small particles first formed diffuse outward in the capillaries until they aggregate into larger particles, clog up the capillary and so prevent the further diffusion of the other particles. This leads to the formation of narrow ring zones, sometimes very characteristic, in which a reaction product is localized. Such a concentration of small amounts of precipitate on a small surface enhances of course the visibility and thereby increases the sensitivity of detection; it has no equal in the reactions carried out in the test tube, and is the reason why the same chemical reaction carried out in the form of a spot reaction, despite the use of smaller amounts of liquid, may be more sensitive and permit the identification of smaller quantities of substance than a test in a test tube.

The spot plates are of advantage when color reactions are carried out, since colors and color changes are easily observed against the white background of such a plate, especially when the test itself and a blank test are carried out in adjoining depressions in the plate. Non-porous bases differ from filter paper in that they permit the use of any strongly acid or alkaline test solution as well as the addition of more drops, which, in the case of the paper, would result in too large a spreading-out of the spot.

The so-called spot paper No. 601 of Schleicher and Schüll (Düren and New York) may be recommended as the paper best suited for our purposes. It rapidly absorbs the drop placed upon it, without permit-

ting it to spread out as much as on soft paper. For some reactions, however, this paper is not applicable, since it still contains small amounts of impurities (iron, calcium, magnesium, phosphate, silicic acid). In such cases, the quantitative filters (Schleicher and Schüll No. 589), which have only a very small ash content, are to be preferred.

The filter paper may be kept in the usual round or rectangular form or in squares (2 by 2 cm.); it is advisable to keep it in so-called Petri dishes. Impregnated filter papers are for the most part prepared by laying strips of quantitative filters in sufficiently concentrated reagent solutions and subsequently drying them by hanging in a heated drying chamber. It is also possible to add the reagent to the paper by spraying.

The removal and addition or combination of drops of the test and reagent solutions can be effected in a simple way by allowing the proper solution to drop off glass rods (about 20 cm. long and 3 mm. thick). Such drops have on the average a volume of 0.05 cc. Thinner glass rods (1 mm. thick) permit the removal of smaller amounts of liquid. Glass pipets about 20 cm. long which can easily be made by drawing out glass tubes of 4-mm. bore over the flame are recommended. Very small and always uniform droplets may be removed from solutions by means of micropipets or platinum wire loops and brought on suitable bases by dropping, rubbing or flipping off. With platinum wire loops an approximately exact calculation of the amount of liquid added can be made by varying the loop size and standardizing (by weighing a flipped-off water drop). Such loops are easily made by bending platinum wires of varying thicknesses in a circle and soldering. These wires, which are also used in decomposition operations, are fused as usual into glass rods or tubes which can be inserted in hard-glass test tubes by means of cork or rubber stoppers. A note on the drop size of the liquid in question is expedient.

Glass rods and pipets are always kept at hand in sufficient number (20 to 30 pieces); they are kept best by standing (narrow end downward) in a porcelain beaker about 15 cm. high which holds 20 to 30 pieces.

Drops of the reagent solutions are taken from a dropping bottle or better still a pipet bottle; such bottles have the advantage that larger amounts of reagent solutions can be kept ready in proper concentration and free from dust. The reagent bottles of about 50 cc. capacity, as well as vials for the solid reagent and reagent papers, are kept in sufficient number on shelves over the work tables.

If heating is required in spot reactions for bringing about complete conversion, the paper with the added drop may be held over a microflame; placing the paper on the mica chimney of a microburner is also

of advantage. Wherever feasible, however, drop reactions in which heating is necessary are carried out in micro porcelain crucibles or on watch glasses which are placed on a previously heated asbestos plate or wire gauze, or are warmed directly over the free flame by placing the crucible or the watch glass on a clay triangle. Glass rods drawn out to a capillary thickness or platinum wires are used for stirring.

Several characteristic spot reactions are described in the following.[1]

2. Selected Examples

Exercise 77. Detection of Copper with Rubeanic Acid [1]

An alcoholic solution of rubeanic acid (diamide of dithio-oxalic acid) which in the so-called aci-form $HN{=}C{-}C{=}NH$ (SH SH) is capable of forming salts, produces a black precipitate of copper rubeanate in ammoniacal or weakly acid copper solutions.

A drop of the neutral test solution is placed on filter paper, exposed to ammonia fumes and touched with a drop of the reagent solution; depending upon the quantity of copper, a black spot or ring appears.

It may be remarked that the minute traces of copper in distilled water will give a reaction of their own under the conditions of the experiment. Therefore, for more certain recognition of very small quantities of copper, a blank experiment is necessary.

Limit of identification: 0.006 γ copper. (With the use of microdroplets [0.015 cc.].)

Limit concentration: 1 : 2,500,000.

Reagents: 1. Ammonia.

2. One per cent alcoholic solution of rubeanic acid.

If it is desired to detect copper in the presence of cobalt and nickel salts, which react at the same time with rubeanic acid to form brown-yellow or violet compounds, use may be made of the fact that, on addition of a drop of test solution to the reagent-impregnated paper, a capillary separation of the different-colored rubeanates results.[2]

Using a neutral test solution containing copper and cobalt, a central black or dark brown ring of copper rubeanate surrounded by a yellow concentric ring (cobalt rubeanate) is obtained. In this way 0.05 γ copper is still identifiable in the presence of 20,000 times as much cobalt.

[1] The journal Mikrochemie will shortly contain a bibliography of spot analysis in which a color chart will be included.

[1] Pr. Ray, Z. anal. Chem. **79**, 94 (1929); see also Pr. Ray and R. M. Ray, J. Indian Chem. Soc. **3**, 118 (1926); Chem. Abstracts, **20**, 3690 (1926).

[2] F. Feigl and H. J. Kapulitzas, Mikrochemie **8**, 239 (1930).

If copper is to be detected in the presence of nickel, the test solution is first acidified with acetic acid and only then a drop placed on the reagent paper; in the middle of the spot a dark ring of copper rubeanate forms, while the nickel diffuses further and forms a violet or blue ring around the central one. The blue ring moves toward the center after standing for a time, owing to the evaporation of the acetic acid.

Limit of identification: 0.05 γ copper } in the presence of 20,000
Limit concentration: 1 : 1,000,000 } times as much copper.

Concerning the foregoing tests, it must be remarked that the cobalt and nickel solutions which are to be tested for copper should not be stronger than 2 per cent in respect to these two metals.

Exercise 78. Detection of Bismuth with Alkaline Stannite Solution in the Presence of Lead Salts [1]

Lead salts are only very slowly reduced by alkaline stannite solutions at room temperature. For example, if a drop of 1 per cent lead acetate solution is treated on the spot plate with a drop of stannite solution it becomes light brown only after 3 to 10 minutes, owing to the separation of lead.

The reaction

$$Pb(OH)_2 + Na_2SnO_2 \rightleftarrows Pb + Na_2SnO_3 + H_2O$$

which proceeds only slowly by itself, is accelerated extraordinarily by the simultaneous precipitation of bismuth. Quantities of bismuth too small to be identified by themselves by reduction with an alkaline stannite solution have this effect. Apparently the bismuth traces catalyze the lead reduction by functioning as crystallization nuclei for the precipitated lead. This fact permits the identification of very small amounts of bismuth by the induced reduction of the lead.

In the acid sulfide group, the detection of bismuth is definite in the absence of silver, copper and mercury; the interference of the last two metals is easily eliminated.[1]

A drop of the test solution (acid with hydrochloric acid if possible), a drop of a saturated lead chloride solution and two drops of stannite solution are brought together on a spot plate and mixed. In the presence of larger amounts of bismuth a precipitate of metallic lead forms immediately; with smaller amounts, a light brown color develops after 1 to 3 minutes which gradually deepens and finally leads to complete precipitation of the lead. Since lead salts themselves are reduced, although slowly, it is always advisable to make a comparison test with

[1] F. Feigl and Krumholz, Ber. dtsch. chem. Ges. **62**, 1138 (1929); see also F. Feigl, Qualitative Analyse mit Hilfe von Tüpfelreaktionen, Akad. Verl. Ges. Leipzig, 1930.

a drop each of hydrochloric acid and lead chloride solution and two drops of stannite solution.

Limit of identification: 0.01 γ bismuth.

Limit concentration: 1 : 5,000,000.

Reagents: (1) Stannite solution. This is prepared shortly before use by mixing equal parts by volume of the following solutions: (*a*) 25 per cent sodium hydroxide solution, (*b*) solution of 5 g. stannous chloride in 5 cc. concentrated hydrochloric acid diluted to 100 cc. with water.

Exercise 79. Detection of Nickel with Dimethyl Glyoxime

The sensitivity of the familiar nickel test of Tschugaeff is increased very remarkably when it is carried out as a spot reaction by placing a drop of the test solution on filter paper impregnated with dimethyl glyoxime. It is possible to detect in this way 0.015 γ nickel in one drop, corresponding to a dilution of 1 : 3,330,000, by the formation of a rose ring.

Detection of Traces of Nickel in Cobalt Salts[1]

Small amounts of nickel are not detectable with dimethyl glyoxime in the presence of much cobalt salts because cobalt reacts with the reagent under the conditions of the precipitation with the formation of soluble cobalt dimethyl glyoxime compounds and thereby uses up the reagent. Besides, the nickel dimethyl glyoxime is also markedly soluble in the solution of the cobalt salt. However, nickel as well as cobalt forms soluble complex compounds with potassium cyanide which differ in their behavior toward formaldehyde. The complex cobalt cyanide is unchanged by formaldehyde; on the other hand, the nickel compound is transformed into nickel cyanide with evolution of hydrogen cyanide and can be converted into the familiar red compound with dimethyl glyoxime. In this way the interference by cobalt salts in the nickel detections may be eliminated.

A lentil-sized kernel of the soluble cobalt salt to be tested (about 0.05 to 0.06 g.), as well as an equally large amount of nickel-free cobalt salt, are dissolved in adjacent depressions in the spot plate in one to two drops of water and then treated dropwise with a concentrated potassium cyanide solution with stirring until the precipitate appearing at first is redissolved. Then one to two drops of hydrogen peroxide are added to convert into $K_3Co(CN)_6$, and the solution is allowed to stand with stirring until it has become light yellow, which will require only a few minutes. Then a knife-point of solid dimethyl glyoxime and several drops of formaldehyde are added and stirred up. In the pres-

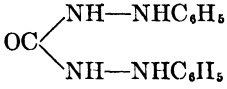
[1] F. Feigl, Mikrochemie, Emich-Festschrift 128 (1930); the preparation of nickel-free cobalt salts is also described here.

ence of nickel the solution turns orange-red, or red nickel dimethyl glyoxime is precipitated. The comparison test with nickel-free cobalt salt remains yellow and permits recognition by comparison of very small color differences caused by traces of nickel.

Reagents: 1. Saturated potassium cyanide solution.
2. Three per cent hydrogen peroxide solution.
3. Formaldehyde solution (40 per cent).
4. Dimethyl glyoxime (solid).

Exercise 80. Detection of Chromium (or Chromate) with Diphenyl Carbazide [1]

Chromates react in acid solution with diphenyl carbazide



with the formation of soluble violet compounds of as yet unknown constitution.

Since chromic salts are easily transformed into chromates by oxidation, the reaction of the latter with diphenyl carbazide presents the possibility of a sensitive test for chromium. A suitable oxidizing agent is alkaline bromine water (hypobromite), which transforms chromic salts very quickly into chromates. Then the excess bromine is removed by addition of phenol, forming tribrom phenol, and is thereby rendered inactive.[2]

A drop of the test solution, acidified with a mineral acid, is treated on the spot plate with a drop of saturated bromine water and then with two to three drops of 2 *N* potassium hydroxide solution; the solution must react alkaline to litmus. After thorough mixing, a little crystal of phenol and a drop of diphenyl carbazide solution are added and then 2 *N* sulfuric acid until the red color due to the alkaline diphenyl carbazide disappears. In the presence of chromate a blue-violet color remains.

Limit of identification: 0.25 γ chromium.

Limit concentration: 1 : 200,000.

Reagents: 1. Saturated bromine water.
2. 2 *N* potassium hydroxide solution.
3. Phenol (solid).
4. One per cent alcoholic diphenyl carbazide solution.
5. 2 *N* sulfuric acid.

Still smaller amounts of chromium may be identified in the following way: A drop of the solution to be tested is evaporated to dryness

[1] P. Cazeneuve, Comp. rend. **131**, 346 (1900); Chem. Zblt. **2**, 688 (1900).
[2] K. Heller and P. Krumholz, Mikrochemie **7**, 220 (1929).

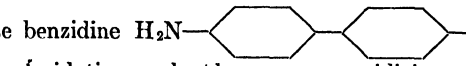
in a micro porcelain crucible and the residue fused with a few milligrams of a mixture of equal parts of sodium carbonate and sodium peroxide. After cooling, the melt is acidified with dilute sulfuric acid and one to two drops of the alcoholic diphenyl carbazide solution added.

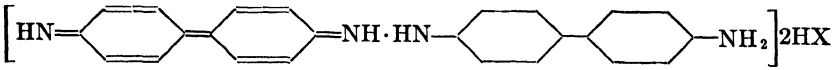
Limit of identification: 0.02 γ chromium.

Limit concentration: 1 : 2,500,000.

The latter procedure also permits the detection of traces of chromium in a few milligrams of finely powdered minerals and rocks.

Exercise 81. Detection of Manganese with Benzidine [1]

The organic base benzidine  may be transformed into a blue oxidation product by numerous oxidizing agents, as well as by auto-oxidation processes. The reaction product is a so-called "semi-quinoid" compound,[2] i.e., a molecular combination of one mol imine and one mol amine with two equivalents of acid with the following formula:



(X = monovalent acid radical)

Since precipitated manganous hydroxide in contact with air is converted according to the equation

$$Mn(OH)_2 + O \rightarrow \underbrace{\begin{matrix} MnO(OH)_2 \\ MnO_2 \cdot H_2O \end{matrix}}$$

into hydrated manganese dioxide, which reacts with benzidine to form the above-mentioned quinoid compound, small amounts of manganese may thus be identified by the color reaction.

A drop of the test solution is placed on filter paper and touched with one drop of dilute alkali and then with one drop of a benzidine solution; a blue color results, the intensity of which is dependent on the amount of manganese present.

Limit of identification: 0.15 γ manganese.

Limit concentration: 1 : 330,000.

Reagents: 1. 0.05 *N* potassium hydroxide solution.

2. Benzidine solution: 0.05 g. benzidine, free base or hydrochloride, dissolved in 10 cc. acetic acid, brought to 100-cc. volume with water and filtered.

Traces of manganese may be detected in drinking water by a spot reaction in the following way: 100 to 150 cc. of the water are treated

[1] F. Feigl, Chem. Ztg. **44**, 689 (1920).

[2] W. Schlenk: Liebig's Ann. **363**, 313 (1908).

with several drops of alkali, boiled, the solution poured through a quantitative filter and washed once with distilled water. On addition of a drop of the benzidine solution to the filter paper, as little as 1.2 γ manganese (1 : 125,000,000) may be recognized by the formation of a blue spot.

Exercise 82. Detection of Magnesium in Tap Water [1]

Magnesium hydroxide strongly adsorbs numerous dyes and is able to take these up with change of color.[2] Thereby very small amounts of magnesium hydroxide are made visible which would otherwise be unrecognizable in a solution.

A drop of tap water is mixed on a spot plate with one to two drops of the alkaline dye solution. Depending upon the amount of magnesium, either a blue precipitate or a color change of the red-violet reagent solution to blue takes place.

If only traces of magnesium are contained in the water (the limit of identification of the reaction is 0.19 γ Mg), then a simultaneous comparison test with a drop of distilled water is recommended.

Reagent: One milligram *para*-nitrobenzol-azo-α-naphthol in 100 cc. 2 N potassium hydroxide solution.

Exercise 83. Detection of Phosphoric Acid with Ammonium Molybdate and Benzidine [1]

Normal molybdates and free molybdic acid are without effect on the organic base benzidine; on the other hand, molybdenum trioxide in a complex compound is able, under proper conditions, to oxidize the benzidine momentarily into a blue quinoid compound (see also manganese) with simultaneous reduction of the molybdenum trioxide to the so-called "molybdenum blue." The simultaneous formation of the two colored reaction products therefore gives a sensitive spot test for phosphoric acid.

A drop of the ammonium molybdate solution is placed on a "blue band filter" (Schleicher and Schüll No. 589) and dried in the drying oven; the freshly impregnated paper is then touched with one drop of the test solution followed successively by one drop of the benzidine solution and a drop of sodium acetate solution. Depending upon the amount of phosphate, either a blue spot or a similar ring forms.

Limit of identification: 0.05 γ P_2O_5.

Limit concentration: 1 : 1,000,000.

[1] F. Feigl, Qualitative Analyse mit Hilfe von Tüpfelreaktionen, 245, Akad. Ver. Ges., Leipzig, 1930.

[2] See also for this E. Eegriwe, Z. anal. Chem. **76**, 354 (1929), and Exercise 74.

[1] F. Feigl, Z. anal. Chem. **61**, 454 (1922); **77**, 299 (1929).

Reagents: 1. Ammonium molybdate solution: 5 g. ammonium molybdate are dissolved in 100 cc. cold water and poured into 35 cc. nitric acid, sp. gr. 1.2.
2. Benzidine solution: 0.05 g. benzidine or benzidine hydrochloride are dissolved in 10 cc. acetic acid and diluted to 100 cc. with water.
3. Saturated sodium acetate solution.

Exercise 84. Detection of Sulfides, Thiosulfates and Thiocyanates with Sodium Azide and Iodine [1]

The reaction between sodium azide and iodine, which by itself proceeds infinitely slowly, is catalytically accelerated by very small amounts of sulfides (soluble as well as insoluble), thiosulfates and thiocyanates. The reaction proceeds as follows:

$$2NaN_3 + I_2 = 2NaI + 3N_2$$

The compounds mentioned may then be recognized by the evolution of nitrogen, which occurs after their addition to a clear solution of sodium azide and iodine-potassium azide.

In the form of a drop reaction, the detection may be carried out by placing a drop of the reagent and the test solution on a little watch glass, which is preferably placed on a dark base. A more or less vigorous evolution of nitrogen, depending upon the amount of the sulfur compound, takes place and can easily be recognized by the little gas bubbles.

The tests made possible by this catalytic reaction are exceedingly sensitive, as the accompanying data show.

Limit of identification: 0.02 γ sodium sulfide (1 : 2,500,000).
0.05 γ sodium thiosulfate (1 : 1,000,000).
0.065 γ potassium thiocyanate (1 : 770,000).

It may be remarked that sulfide solution (prepared freshly by combination of NaOH and H_2S as well as by dissolving solid preparations) always show, even if only a very minute, content of thiosulfate which may be recognized in the following way.

A drop of the solution is brought on a spot plate and stirred with several crystals of cadmium nitrate. A strip of filter paper (0.5 by 4 cm.) is dipped into the suspension of cadmium sulfide thus formed. A capillary rise of the clear liquid with precipitation of cadmium sulfide at the lower edge of the filter strip results. After about 1 to 2 minutes the filter strip is withdrawn from the liquid, cut off above the sulfide fringe and placed on a watch glass. If a drop of an iodine-azide solution is then placed on the moist part of the paper, a definite evolution of nitrogen bubbles takes place. Some of the bubbles remain adhering to the watch glass when the paper is drawn away.

[1] F. Feigl, Z. anal. Chem. **74**, 369, 376 (1928); Mikrochemie **7**, 10 (1929); also M. Niessner, Mikrochemie **8**, 121 (1930); L. Metz, Z. anal. Chem. **76**, 347 (1929).

Appendix I

List of Apparatus

Preliminary Remarks. The apparatus at hand in every chemical laboratory, such as stands, beakers, flasks, test tubes, etc., are not included in this list. Microscope, magnifiers and the like are mentioned only in so far as special requirements come into consideration. Simple glassware, such as centrifuge cones, microbeakers, etc., can be made by the worker himself without difficulty or, if necessary, by any glass-blower, who can easily make such apparatus according to the illustrations. Dr. A. Benedetti-Pichler has listed the most important apparatus mentioned in the "Manual," divided for qualitative and quantitative work. These collections may be obtained from K. Schmitt, Lessingstrasse 25, Graz, in carrying cases which greatly facilitate storage and transport. The schlieren microscope with accessories as well as the Behrens box (with about 120 reagents) may be obtained from the same source. In addition, most of the apparatus not carried by the usual supply houses is obtainable from P. Haack, Vienna IX, Garelligasse 4. For the source of supply of apparatus of F. Pregl, see his "Quantitative Organic Microanalysis." An American source of supply for all microchemical apparatus, both organic and inorganic, is Microchemical Service, 30 Van Zandt Avenue, Douglaston, New York.

The number at the right after each piece of apparatus indicates the page of the "Manual" where it is mentioned.

List A: Necessary Apparatus

List B: Very Desirable Supplement to List A

List C: Desirable Supplement to Lists A and B

1. For the microscope: large stand suitable for projection and photomicrography, camera, case and other accessories, especially silver screen and projection mirror.
Selection of (compensating) eyepieces. Immersion objective (2 mm.). Vertical illuminator. Binocular attachment..
Test objects. Camera lucida. Quartz slide and cover glass for ultramicroscopy. Cover glass for microchemical reactions. Special objective, cardioid condenser, change-over condenser.
Microspectrophotometer.
Hot stage.
Equipment for fluorescence experiments (quartz condenser, Euphos cover

[1] F. Emich, Methoden der Mikrochemie, in Abderhalden's Handbuch I, 3, Vienna and Berlin, 1921.

PAGE

glass, quartz slide, total reflecting prism of quartz for illumination; U–V glass filter, nickel arc lamp, etc.).
Ultra-violet lamp 121
Electrodes and slide for qualitative microelectrolysis 44

2. Finer Nernst balance (see "Methods," pp. 233).
Balance of Steele and Grant or H. Pettersson, Electromagnetic micro balance ("Methods," pp. 193, 206).
Torsion spring balance of Hartmann and Braun, "Methods," p. 352 61
3. Black box for the investigation of turbidity and fluorescence (Böttger, Qual. Anal., Leipzig, 1925, p. 207).
4. Larger selection of quartz apparatus.
5. Microburets according to Pilch ("Methods," p. 308), or Bang (Mikromethoden z. Blutuntersuchung, Munich, 1922); or Benedetti-Pichler or Schilow (e.g., Z. anal. Chem. **73**, 200 [1928]. Weighing burets.
6. Gas analysis apparatus, such as the apparatus of Guye and Germann ("Methods," p. 315); of A. Krogh (Skand. Arch. Physiol. **20**, 279 [1907]; or of J. A. Christiansen (Z. anal. Chem. **80**, 435 [1930]).
7. Interferometer, refractometer, spectral apparatus, colorimeter, nephelometer according to Kleinmann, stupho-photometer.
Apparatus for potentiometric determinations, micromanipulator and tools, etc.

Appendix II

Sundry Very Simple Apparatus

In order to facilitate the carrying out of microchemical experiments by persons who have only limited facilities at their disposal, several suggestions are given in the following as to how one can do without apparatus which is not available in some institutes. It must be emphasized that these are only makeshifts which should be replaced as soon as possible by the correct equipment.

1. Substitute for *eyepiece micrometer.* (*a*) Since the *accurate measurement of microcrystals* may usually be replaced by an estimation of their size, the following set-up will be sufficient.

A ruled sheet (such as usually furnished with writing tablets; called a "Faulenzer" in Austria) is placed beside the microscope in such a way that it is 25 cm. (distinct range of vision) from the eye (which is not looking into the microscope); since the microscope is usually higher, the sheet is placed on several books. We will assume that the lines on the sheet are 1 cm. apart. If the magnification of the system with which we are working is known, the microscopic picture need only be measured off on the ruled sheet and divided by the magnification. If the magnification is not known, it is determined by means of the same set-up, using, however, a millimeter (or half-millimeter) scale as object. With the low-power system, several lines drawn with a drawing pen or well-sharpened pencil will suffice. For the higher-power system, however, such a scale will hardly do. In the latter case, a microscopic object such as a crystal is first measured with the low magnification and then calibrated with the high magnification and ruled sheet. Of course the sheet need not be at a distance of exactly 25 cm. The value of each division can also be determined for any other distance.

(*b*) This method of working is hardly sufficient for the Barger molecular weight determination, but the ingenuity of the reader will find a substitute.

2. The *rotating stage* is replaceable by the rotating slide. That is, a ring (of metal, cardboard or cork) which fits in the circular opening in the stage below the object, is fastened to the lower side of the slide with marine glue or sealing wax.—If angles are to be measured (see exercise with gypsum, p. 101) a sheet of paper with a circular hole in the center is laid on the stage and is fastened to it with, e.g., some adhesive wax (resin and wax mixture). The slide is turned until a crystal edge is exactly parallel to a comparison line (table edge, edge of paper, ruled sheet) which is observed with the second (free) eye. Then a pencil line is drawn along the edge of the slide. The crystal is rotated until the second edge is parallel to the same line, and another pencil line is

drawn. The angle obtained is measured with a protractor.—Often it will be advisable to use the rotating slide as a base for the real object slide.

3. *Substitute for Nicol Prisms.* (*a*) The *polarizer* can be replaced by various mirrors. For example, a black glass plate (see below) may be placed on the illuminating mirror and inclined so that it forms an angle of about 33° with the axis of the tube. Light from a mirror lying almost horizontal on the table is allowed to fall on the glass plate at an angle of 33° to the horizontal. The apparatus shown in Fig. 86 [1] is more convenient. A cardboard or wooden frame contains an amalgam mirror *G* and a black mirror *S* inclined 33° to the horizontal. The latter is made by painting a blank, well-cleaned mirror glass plate on the reverse side with black asphalt lacquer. The mirrors should be twice as long as wide, 7 by 14 cm. is more than sufficient. The polarized light from *P* is directed to the microscope mirror. The simplest arrangement is to place the "polarizer" on a wooden block, naturally properly inclined, in the path of the light rays, i.e., between the source of light and the illumination mirror. (*b*) For *analyser*, a pile of 12 to 20 cover glasses which are fixed in a longitudinally bored cork at an angle of about 57° to the horizontal (Fig. 87) may be used. The interior of the cork is painted black. The cork is cut apart along the plane *AB*, with a notch *DEFG* cut out in which the cover glasses are placed, and then glued together again. *HJ* is a diaphragm made of black cardboard with circular opening in the center.—The "analyser" is to be placed on the eyepiece of the microscope. In order to prevent it from easily falling off, a rim *R* of paper is placed on the lower edge so that it fits over the eyepiece. This apparatus naturally does not give complete darkening but permits the observations necessary for our purposes.

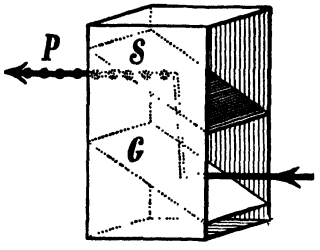


FIG. 86.—Substitute for polarizer.

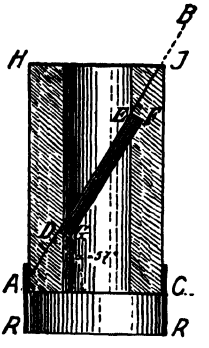


FIG. 87.—Substitute for analyser.

4. *Dark Field Illumination.* Several little opaque plates, 2 to 3 mm. in diameter are made, e.g., by hammering small shot kernels flat. Naturally the diameter of the plate must fit the aperture of the objective, therefore a certain selection of the plates is necessary. The plate is laid on the condenser, which is lowered sufficiently to prevent the slide above it from touching it. The slide is removed and the plate centered by looking into the microscope (low magnification) and moving the plate into the proper position with a pointed splinter. The light used for these operations must not be too bright. Then the ultra object, e.g., a colloidal gold

[1] K. Rosenberg, Experimentierbuch für den Unterricht in der Naturlehre, Vol. 2, p. 497, Vienna and Leipzig, 1913.

solution, is put under the microscope, a correspondingly stronger source of light as well as higher magnification being used. Instead of the metal plates, a number of round spots may be made on the lower side of the slide with asphalt lacquer, only in this case it is not possible to move the object unless two thin slides are used, the lower one holding the opaque plates.

5. In place of the experiment with liquid carbon dioxide given on p. 14, a medium thick capillary tube can be filled with ether and sealed shut so that a sufficiently long handle remains attached and so that the real bomb is half filled. The meniscus vanishes in nitrobenzene vapor. Caution because of the pressure of about 40 atmospheres! See also Physik. Zeits. **17**, 454 (1916).

6. The *centrifuge* may—of course only in extreme need—be replaced by a wooden block which is swung in a circle by a very strong string. A hole is bored in the block to hold the tubes which are to be centrifuged. For a very simple substitute, see also the paper of F. L. Hahn.[2]

7. The following may be mentioned in reference to the manufacture of *cells* for schlieren observations. The cells consist of three parts, the slide, the center or form piece and the cover. All three parts may be made of slides 1 to 3 mm. thick by proper cutting. In the case of the round cells, the form piece is cut out with a rotating copper tube, the neck of the cell by a small Carborundum wheel such as used by dentists. The copper tube is well wetted with turpentine, and medium-fine emery powder is added during the grinding. In making the heart-shaped cells the slide is bored in the same way and then ground out to the proper shape with the Carborundum wheel. It is very much simpler if the center piece is made in two parts. (See Fig. 88*b*.) For difficultly volatile and non-hydroscopic such as aqueous liquids, a V-cell may be used, the central piece of which is shown in Fig. 83*a*.

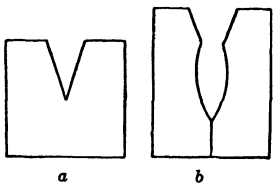


FIG. 88.

Canada balsam, "cololith," guttapercha paper (from the druggist or photographic supply dealer) and silver chloride (melting-point 445°) are used for cementing the three parts together. A glue mixture will also do for hydrocarbons. The cements are spread in a thin layer on both sides of the form piece, the three pieces laid together and the tight and dense juncture made by proper treatment of the cement (e.g., heating on a metal block or in the drying oven). In many cases, it is advantageous to apply pressure to the parts laid together. When using silver chloride or glue, care must be taken to have the glass surfaces which are to be cemented, well cleaned (free from grease).

[2] Mikrochemie Pregl-Festschrift, 127 (1929).

INDEX